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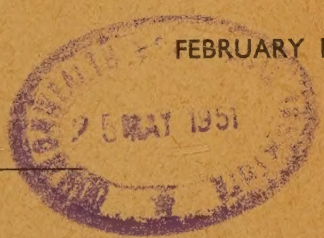

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VOLUME 31, No. 5, 1950

(AGRICULTURAL RESEARCH SECTION)

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NOTICES

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THE NEW ZEALAND

JOURNAL OF SCIENCE

AND

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Department of Scientific and Industrial Research, Wellington

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NOTE ON " AVAILABLE " COPPER IN NEW ZEALAND SOILS

By A. C. S. WRIGHT and H. W. JOHNSTON, Soil Bureau, Department of Scientific and Industrial Research, Wellington

A PROGRESS REPORT ON A SURVEY OF "AVAILABLE" COPPER IN NEW ZEALAND SOILS.

A number of soil types examined by the pot culture technique in 1946 for trace element deficiency showed a response to copper supplied as copper sulphate at rates equivalent to 5 lb. per acre. As no analyses of plant material were made and as copper salts are known to have fungicidal properties, it seemed desirable to find out whether the soils in question were actually deficient in assimilable copper.

Chemical methods for estimation of assimilable or "available" copper are not always reliable when a wide range of soils, both mineral and organic, is involved (1). It was therefore decided to try a bio-assay method employing *Aspergillus niger,* and following the technique described by Mulder (2). Dr. Mulder kindly supplied cultures of the strain of *Aspergillus* used in his original researches.

The method has been satisfactorily adapted for use with New Zealand soils and reproducible results have been obtained with fresh or stored (dried or refrigerated) soil samples.

Of the ten selected virgin topsoils examined to date, four have a very low content of available copper, and two soil types (Te Kopuru sand and Wharekohe silt loam) are known to respond in pot culture experiments. Two other soils have but slightly better "available" copper status; and in only one topsoil culture did the colour of the fungus indicate an uptake of copper greater than 4 p.p.m. (Table I.)

These results confirm the possibility of there being a copper deficiency in some of the more leached soils of North Auckland; and also indicate that "available" copper is less than 4 p.p.m., in some organic soils. There is no report of any marked copper deficiency symptoms appearing in either plants or animals on the North Auckland "gumlands" or similar related soils, but on many organic soils throughout New Zealand, stock are unthrifty and suffer ailments which can be ameliorated by treatments with copper salts (3).

(1) ACOCK, A. M. (1941) : *J. Coun. Sci. Ind. Res., 14,* No. 4, 288-300.

(2) MULDER, E. G. (1939-40) : *Antoine van Leevwenboek, 6,* 99-109.

(3) CUNNINGHAM, I. J. (1944) : *N.Z. J. Agr.,* 68-69, 559-569.

TABLE I. "AVAILABLE" COPPER IN VIRGIN SOIL SAMPLES

Lab. No.	Depth. (in.)	Soil Type.	Classification.	Locality.	Available Copper. (p.p.m.)
3882	0– 6*	Te Kopuru sand	Podzol from consolidated sand	Kaihu, North Auckland	0·6
4825	0– 6†	Te Kopuru sand	Podzol from consolidated sand	Kaihu, North Auckland	0·3
4420	0– 6†	Wharekohe silt loam	Podzol from claystone	Purya, North Auckland	0·7
4829	0– 42†	Red Hill sand	Recent and young soils of coastal dunes	Ruakaka, North Auckland	2·1
4828	0– 2†	Marsden sand	Recent and young soils of coastal dunes	Ruakaka, North Auckland	0·6
4827	0– 5†	Marsden peaty sand	Organic soils (peat and blown sand)	Ruakaka, North Ackalnd	0·3
4826	0– 5†	Marsden peaty sandy loam	Organic soils (peat and blown sand)	Ruakaka, Norrh Auckland	1·0
4819	0– 5†	Ruakaka peaty sandy loam	Organic soils (peat and blown sand)	Ruakaka, North Auckland	0·8
(4893A	0– 6†	Ngatea peaty loam	Organic soils (shallow peat estuarine clays)	Kerepuhi Hauraki Plns.	3·7
(4893B	10–15†				3·1
(4864	0– 4†	Piako peaty sandy loam	Organic soils (peat with pumice)	Waitakaruru Hauraki Plns.	2·0
(4863	4– 9†				>4·0
(4868	0– 4†	Rukuhia peat	Organic soils (deep peat)	Waitakaruru Hauraki Plns.	
(4865	4–14†				
4894	0– 8*	Rukuhia peat	Organic soils (deep peat)	Waitakaruru, Hauraki Plns.	>4·0

* = Composite sample. † = Pit sample.

EVALUATION OF THE CHILLER BEEF CARCASS

By H. KNEEBONE, Auckland Farmers' Freezing Co. Ltd., T. MARKS, Auckland Meat Co. Ltd., C. P. MCMEEKAN and D. E. WALKER, Ruakura Animal Research Station, Department of Agriculture, Hamilton

Summary

As an aid to carcass competition work, and to provide a basis for evaluating as objectively as possible the chiller beef carcass in experimental work, a system of judging is presented.

Seventy per cent of the points are awarded on a basis of measurement and thirty points on eye judgment. The details of the system are described and illustrated.

The organization of the system in competitions and under commercial slaughtering conditions is described.

Results obtained under the system have proved very satisfactory as judged by the opinions of local and United Kingdom commercial meat specialists.

INTRODUCTION

THE marked success of the New Zealand judging system for pork and bacon pig competitions (McMeekan *et al.*, 1944) and general pig improvement work has encouraged the authors to extend the principles involved to the evaluation of chiller beef carcasses. Little organized effort has been made in New Zealand to educate producers as to chiller beef requirements. The desirability of making beef production as efficient as possible emphasizes the need for improvement measures.

As in the development of the system now commonly used with pigs, the chief objectives have been threefold—

(1) The elimination of "eye judgments" as far as possible in favour of objective assessments based on measurement.

(2) The standardisation of judging methods so that evaluations are uniform and independent of districts, judges and years.

(3) The scoring of every carcass, independent of its quality or final position in a competition, so that an individual, factual report is available to the fattener and breeder.

Through the co-operation of A. & P. Associations, Freezing Companies and Meat Trade experts, opportunity was taken to obtain measurements of several hundred chiller beef carcasses exhibited at various Shows during the last four years. With the data thus available, along with comparable information from beef cattle exhibited at the Smithfield Fat Stock Shows over many years (Hirtzel, 1939), the system described has been evolved. The system has been tested at several official competitions as well as with a large number of small lines of commercial carcasses. The general aim has been to arrive at a system which sorted out carcasses in the same order as when placed by eye by highly skilled and experienced beef judges. As a final test four separate lines of competition chillers were judged under the system and then shipped to Smithfield Market, England, for expert evaluation by representatives of the Trade and by Dr. John Hammond of Cambridge. The results of these tests have been so successful as to encourage the authors to present the system in the hope that it may be widely adopted within New Zealand for chiller beef competition work. In

addition, it should be of value to research workers in the chiller beef field. It has been used at Ruakura for this purpose. The system has been in use in the Waikato-Auckland area for four years and in the opinion of professional Meat Trade interests has led already to a marked improvement in the type of carcass coming forward. Farmers have appreciated its simplicity and the fact that for the first time they are able to see just where their animals do not measure up to the required standards.

JUDGING STANDARDS

The following table shows the distribution of points:—

TABLE I. N.Z. CHILLED BEEF JUDGING STANDARDS: SCALE OF POINTS

	Points	
By Measurements:		
1. Fullness of Meat (Eye muscle depth) B.	20	
2. Fat Cover (Depth over eye muscle) C + D	15	
3. Blockiness (Length of leg in crutch) T	20	
4. Balance of Carcass (Fore-end - Hind-end weight) ..	10	
5. Weight suitability	5	
	—	70
By Eye Judgment:		
6. Rib cover (Muscle and Fat) (5) and Evenness of Fat Distribution (10)	15	
7. Colour and Texture of Muscle	5	
8. Colour and Texture of Fat	5	
9. Marbling of Muscle (Finish)	5	
	—	30
TOTAL		100

It will be noted that 70 per cent of the total points are awarded on a basis of measurement and 30 per cent on eye judgement. It has not been possible to devise satisfactory methods of measuring the characters involved under the latter head.

The system emphasizes the major importance of muscle development, blockiness and fat cover in the beef animal. It is fortunate that the normal method of quartering sides of beef for transport permits an accurate measurement of muscle development on a cut surface in the most valuable region of the carcass. Fat cover is also measured in this same region. Considerable emphasis is placed on a general weakness in New Zealand beef, viz., the tendency for beasts to be heavier in fore-ends than hind-ends. Weight suitability is taken into account since weight itself is an important quality factor in the modern meat market. In addition, all tables are scaled for weight, the optimum measurement required being related to the weight of the carcass. The other qualities considered are self-explanatory with the possible exception of "blockiness" which is measured by the length of leg on the hind-end. A short leg measures not only the conformation of the hind-end but is a very good index of blockiness throughout the carcass.

APPLICATION OF JUDGING STANDARDS

The following description, diagrams and tables should permit any experienced beef judge to apply the system.

1. *Fullness of Meat (Eye Muscle Depth)—20 points*:

The eye muscle depth is measured at the cut surface of the longissimus dorsi between the tenth and eleventh ribs. The measurement (B) is taken at the deepest part of the muscle at right angles to a line joining the main axis of the eye (Fig. 1). Measurements are taken on both fore-quarters. The average of the two measurements is used to award points which are assessed from Table II. It is important to note that the measurement is made on the true "eye muscle" only. No account is taken of the multifidus dorsi, a variable part of which is often seen on the loin cut. This muscle sometimes tends to enfold the corner of the eye muscle near the spinous process of the vertebrae. Though giving the appearance of a very meaty joint, this muscle is so variable that its area in cross section often changes quickly within a very short distance, so that a slight variation in cutting can provide a very different picture on the same carcass. In contrast, the true eye muscle—the longissimus dorsi—is a very much more uniform and reliable index of muscle development.

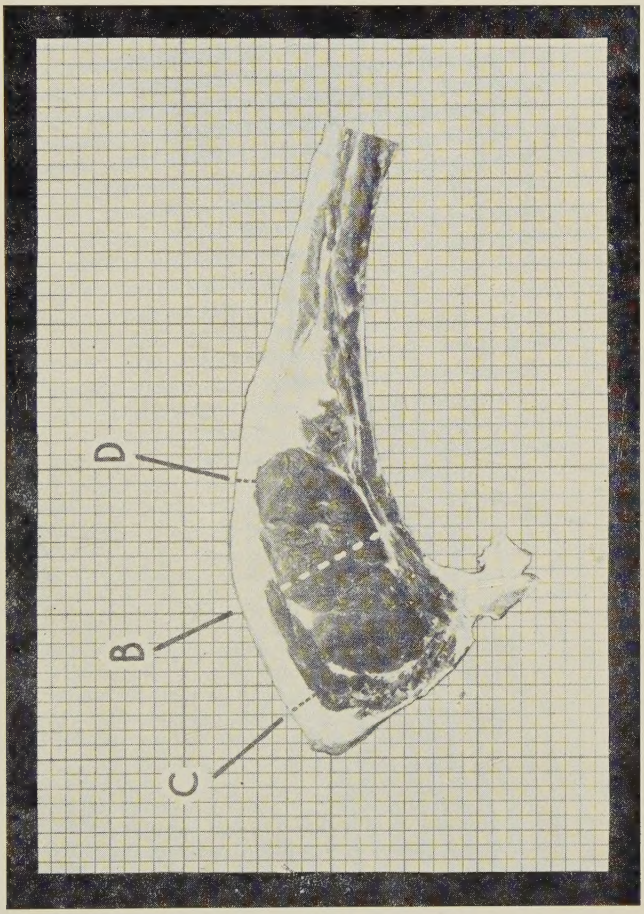

FIG. 1.

TABLE II. FULLNESS OF MEAT—(EYE MUSCLE DEPTH)
(20 Points)
(Measurements in Millimetres)

Weight. (lb.)	Points.									
	20	18	16	14	12	10	8	6	4	2
450 - 475	66	63	60	57	54	51	48	45	42	39
476 - 500	67	64	61	58	55	52	49	46	43	40
501 - 525	68	65	62	59	56	53	50	47	44	41
526 - 550	69	66	63	60	47	54	51	48	45	42
551 - 575	70	67	64	61	58	55	52	49	46	43
576 - 600	71	68	65	62	59	56	53	50	47	44
601 - 625	72	69	66	63	60	57	54	51	48	45
626 - 650	73	70	67	64	61	58	55	52	49	46
651 - 675	74	71	68	65	62	59	56	53	50	47
676 - 700	75	72	69	66	63	60	57	54	51	48
701 - 725	76	73	70	67	64	61	58	55	52	49
726 - 750	77	74	71	68	65	62	59	56	53	50

2. *Fat Cover (Depth over Eye Muscle)—15 points*:

On the same cut as above, the depth of fat over the eye muscle is measured in two places (C and D), and the sum of these two measurements is used in awarding points. The positions are one inch in from the point of the muscle at each end. The position is varied if the dressing of the carcass has removed any of the subcutaneous fat at the normal measuring point. The aim in this measurement is to obtain a fair estimate of the average depth of fat in the loin region. Measurements are taken on both fore-quarters and the average of the two sides is used to award the points which are assessed from Table III (Fig. 1).

TABLE III. FAT COVER. (DEPTH OVER EYE MUSCLE.) (C. PLUS D.)
(15 Points)
Measurements in Millimetres

Weight.	Points—too thin.							Max.	Points—too fat.						
	1	3	5	7	9	11	13	15	13	11	9	7	5	3	1
450 - 475	5	7	9	11	13	15	17	19	21	23	25	27	29	31	33
476 - 500	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34
501 - 525	7	9	11	13	15	17	19	21	23	25	27	29	31	33	35
526 - 550	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
551 - 575	9	11	13	15	17	19	21	23	25	27	29	31	33	35	37
576 - 600	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38
601 - 625	11	13	15	17	19	21	23	25	27	29	31	33	35	37	39
626 - 650	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40
651 - 675	13	15	17	19	21	23	25	27	29	31	33	35	37	39	41
676 - 700	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42
701 - 725	15	17	19	21	23	25	27	29	31	33	35	37	39	41	43
726 - 750	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44
751 & over	17	19	21	23	25	27	29	31	33	35	37	39	41	43	45

3. *Blockiness (Length of Leg in Crutch)—20 points*:

This measurement is made from the top of the tibia when the hind-quarter is in a hanging position (distal end of tibia) to the cut edge of the fat in the crutch. The measurement is made with a steel tape along a line joining the top of the tibia and the H-bone. The point on the tibia from which the measurement is taken is the middle of the easily observed ridge near the distal end of the tibia which is never sufficiently covered with fleshy tissue to be difficult to locate (Fig. 2). Points are awarded according to Table IV. Fig. 3 shows a top scoring and low scoring buttock.

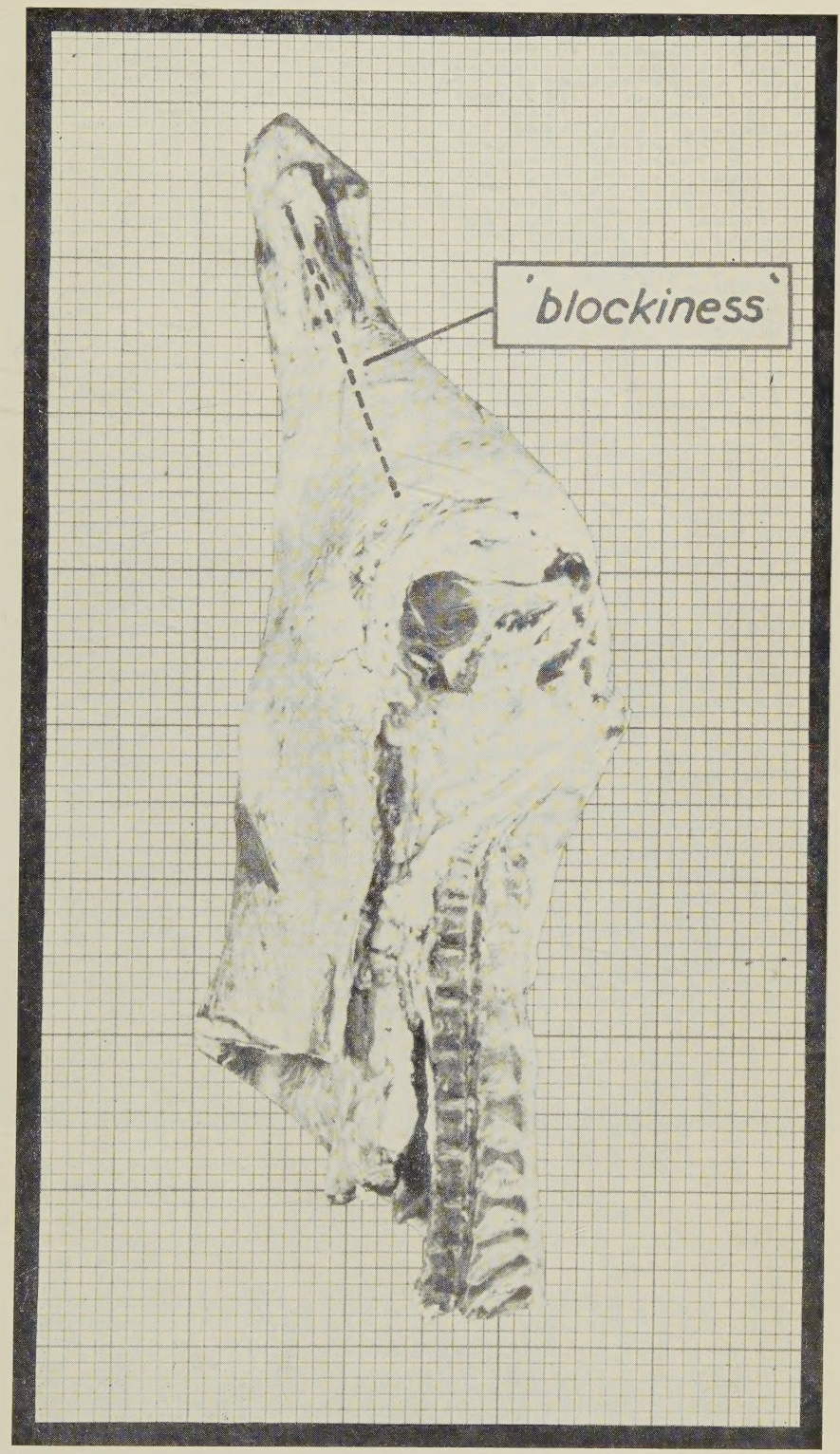

FIG. 2

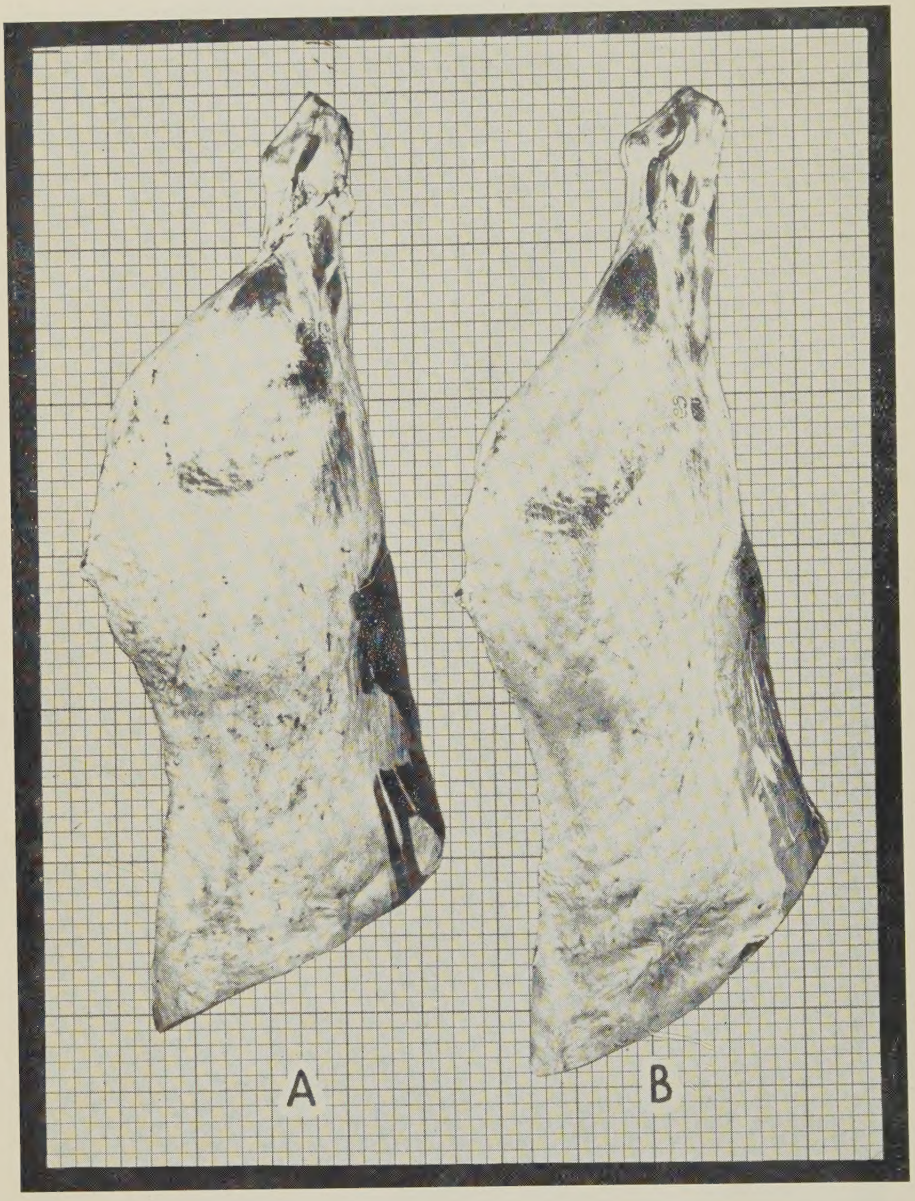

FIG. 3.—A = High scoring buttock. B = Low scoring buttock. Note how measurement system sorts out well-filled, compact, and meaty hind-end.

4. *Balance of Carcass (Difference in Weight between Fore-ends and Hind-ends)—10 points*:

The difference between the total weights of the two fore-quarters and the total weights of the two hind-quarters is obtained. The principle operating is to deduct points where the fore-quarters are heavier than the hind-quarters. The scale used is shown in Table V. As the weight of the carcass increases, hind-ends must be proportionately heavier to

gain full points. An example of a badly balanced carcass with excessive fore-end weight is shown in Fig. 4 in comparison with a well balanced side.

5. *Weight suitability—5 points*:

These are awarded in accordance with Table VI to stress the importance of optimum weight.

TABLE IV. BLOCKINESS—(LENGTH OF LEG IN CRUTCH)
(20 Points)
(Length from Point of Tibia to Fat Edge in H. Bone line
Measurements in inches and quarter inches)

	20	18	16	14	12	10	8	6	4	2
450 475	12^3	13^1	13^3	14^1	14^3	15^1	15^3	16^1	16^3	17^1
476 - 500	13^0	13^2	14^0	14^2	15^0	15^2	16^0	16^2	17^0	17^2
501 - 525	13^1	13^3	14^1	14^3	15^1	15^3	16^1	16^3	17^1	17^3
526 - 550	13^2	14^0	14^2	15^0	15^2	16^0	16^2	17^0	17^2	18^0
551 - 575	13^3	14^1	14^3	15^1	15^3	16^1	16^3	17^1	17^3	18^1
576 - 600	14^0	14^2	15^0	15^2	16^0	16^2	17^0	17^2	18^0	18^2
601 - 625	14^1	14^3	15^1	15^3	16^1	16^3	17^1	17^3	18^1	18^3
626 - 650	14^2	15^0	15^2	16^0	16^2	17^0	17^2	18^0	18^2	19^0
651 - 675	14^3	15^1	15^3	16^1	16^3	17^1	17^3	18^1	18^3	19^1
676 - 700	15^0	15^2	16^0	16^2	17^0	17^2	18^0	18^2	19^0	19^2
701 - 725	15^1	15^3	16^1	16^3	17^1	17^3	18^1	18^3	19^1	19^3
726 - 750	15^2	16^0	16^2	17^0	17^2	18^0	18^2	19^0	19^2	20^0
751 & over	15^3	16^1	16^3	17^1	17^3	18^1	18^3	19^1	19^3	20^1

TABLE V. WEIGHT DIFFERENCE (IN LB.) BETWEEN FORE-ENDS AND HIND-ENDS
(10 Points)

Weight. (lb.)	Points.							
	10	9	8	7	6	5	4	3
450 - 500	+ 5	+10	+15	+20	+25	+30	+35	+40
501 - 550	0	+ 5	+10	+15	+20	+25	+30	+35
551 - 600	− 5	0	+ 5	+10	+15	+20	+25	+30
601 - 650	−10	− 5	0	+ 5	+10	+15	+20	+25
651 - 700	−15	−10	− 5	0	+ 5	+10	+15	+20
701 - 750	−20	−15	−10	− 5	0	+ 5	+10	+15
751 & over	−25	−20	−15	−10	− 5	0	+ 5	+10

TABLE VI. WEIGHT SUITABILITY
(5 Points)

Baby Beef and Heifer Beef.		Chiller Beef.	
Weight. (lb.)	Points.	Weight. (lb.)	Points.
440 - 450	1	500 - 520	1
451 - 460	2	521 - 540	2
461 - 470	3	541 - 560	3
471 - 480	4	561 - 580	4
481 - 520	5	581 - 640	5
521 - 530	4	641 - 660	4
531 - 540	3	661 - 680	3
541 - 550	2	681 - 700	2
551 - 560	1	701 - 720	1

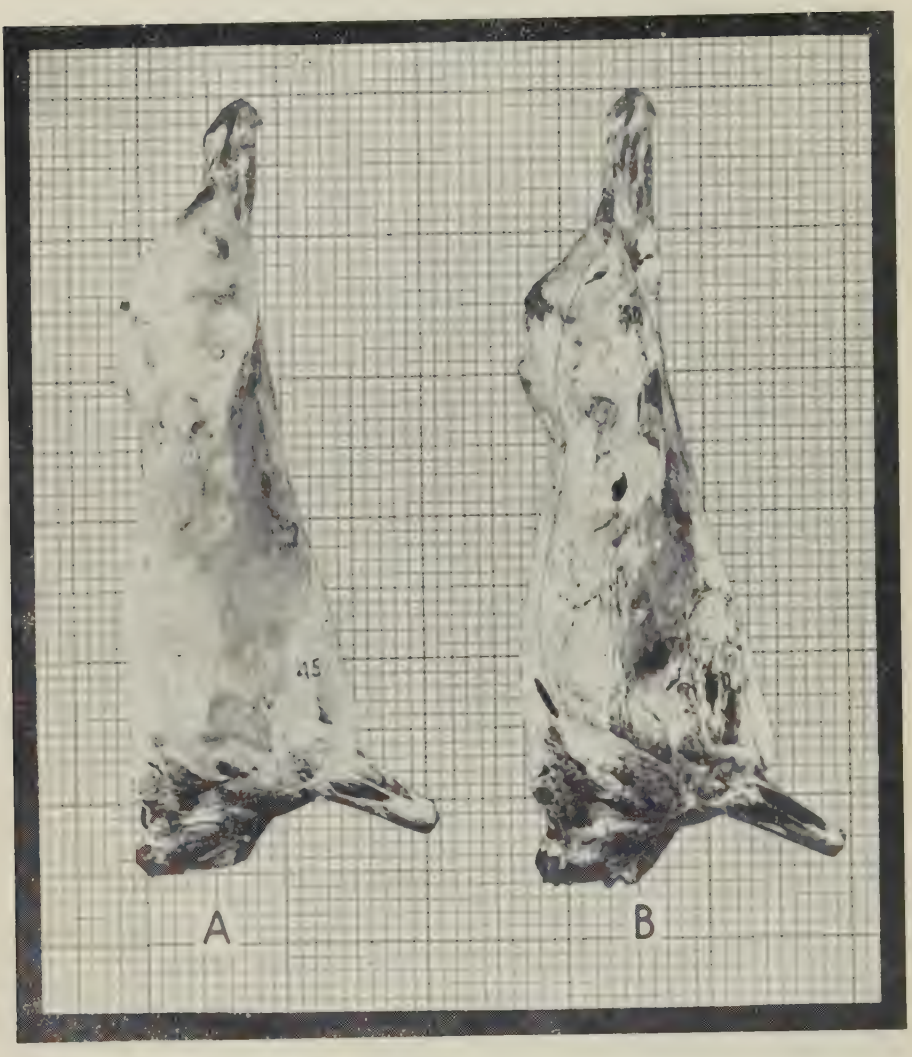

FIG. 4.—A = Good type commercial side showing desirable balance between fore and hind—fair finish.
B = Bad type side showing heavy fore relative to hind—also poor finish.

6. *Rib Cover (Muscle and Fat) and Evenness of Fat Distribution—15 points*:

These are awarded by eye judgment on both sides of the carcass. The rib cover (5 points) is judged on the cut surface between the tenth and eleventh rib which must show an adequate thickness of fleshing over the bone on this surface and a suitable admixture of fat. Evenness of fat distribution (10 points) is judged both on this surface and by observing the fat cover over the whole carcass. Points are deducted for too much or too little fat on the rib cut, insufficient cover over the points, or excessive cover in any part. Fig. 5 shows a high scoring rib cover along with a low scoring (over-fat) and low scoring (under-fat) cover. Excessive kidney, channel or brisket fat is penalized.

FIG. 5.—A = Thick rib cover with good distribution of muscle and fat—4 points. Slight weakness at "a." Note well developed, high-scoring eye muscle—17 points, and fat cover—14 points.
B = Very thick rib cover but excessively fat—2 points. Note poor eye muscle—8 points, and heavy fat cover—6 points.
C = Very thin rib cover, over lean—1 point. Note fair eye for weight—13 points, but insufficient fat cover—6 points.

7. *Colour and Texture of Muscle—5 points*:

This is judged by eye and touch in accordance with normal commercial practice, points being gained for a bright colour and fine texture, deductions being made for a dark colour and coarse texture.

8. *Colour and Texture of Fat—5 points*:

This is judged by eye. The requirement is for a firm, pale fat. Deductions are made for any tendency towards softness or yellow colouration.

9. *Marbling of Muscle—5 points*:

This is judged on the eye muscle on which the measurements have been made. Adequate distribution of marbling fat within the eye muscle is necessary for the maximum marks. Over-finish as well as under-finish is penalized. Ideal finish here is best described as "peppered," the fat being distributed through the muscle as from a pepper pot.

Deduction of Penalty Points:

Occasionally carcasses are found which, on account of some abnormality impossible to measure, are not pleasing to the trade. Obviously the possibilities under this head are too varied to be listed and it must be left to the judges discretion to penalize such carcasses. To meet this situation a deduction of a maximum of five points as a penalty is employed.

Organization of Judging

The adoption of any judging system of this type involves considerably more work than mere placement by eye. All carcasses have to be dealt with in detail and just as much attention devoted to the worst as to the best. Though this is so, we are of the opinion that the results are well worth the extra labour and trouble involved. Difficulties here can be considerably reduced by efficient organization.

From our experience the following recommendations are made:

Three characters are best judged as the animals are dressed and are passing along the "chain" on their way to the chill room: length of leg, evenness of fat distribution and colour and texture of fat. Thereafter the carcass should be chilled as quickly as possible to a firm condition (temperature recommended, 34°F. for twenty-four hours) so as to permit accurate quartering and subsequent measuring. It is also important that quartering should be uniformly and efficiently carried out by a competent butcher. Fat measurements in particular are difficult to take accurately on a badly quartered and imperfectly cooled carcass, while the shape and size of the eye muscle displayed can be influenced by bad quartering. Eye muscle length and fat depth are usually measured together. Fat cover over rib, colour and texture of muscle, and marbling of eye muscle are then evaluated in sequence. At this stage, any soft fat carcass is noted, so that points awarded on the fresh carcass for fat colour and texture can be adjusted if necessary. Texture is not always assessable on the freshly killed carcass. Points for weight suitability and balance of carcass are a simple clerical matter of award in the office. The measurements taken are recorded on score sheets which are passed as completed to the office for award of points from the Tables. Placement is thus completely objective, the judges being unaware of the winning entries until the total scores are available. Fig. 6 illustrates the type of carcass selected by the system as being of high quality. The marks awarded for this carcass are shown on the score card, Fig. 7. This carcass gained the most marks at the Morrinsville Beef Competition, 1948, and was placed second at Smithfield. The English judge's comments were as follows: "Splendid type, short in hock, thigh and rump extremely well made, but showing slight weakness in the middle. Fattening not quite finished in the flesh (marbling), which was bright and attractive—a very close second. The proportionate weight of Hinds and Fores extremely good".

FIG. 6.—Winning Carcass. Morrinsville Show, 1948.

In the four competitions from which all entries have been shipped to Smithfield for special report, the top four carcasses in each case under this measurement system of evaluation have been the same as selected by the Smithfield judges as the best in each line. Only minor alterations occurred in placement, the unanimous report of all English judges being to the effect that this system definitely sorts out the type of chiller carcass required by the British Market.

ACKNOWLEDGMENTS

Thanks of the authors are due to the Auckland Farmers' Freezing Co. and the Auckland Municipal Abbatoirs for the provision of facilities for examining adn measuring carcasses, to the Waikato, Morrinsville and Wanganui A. & P. Show Committees for the co-operation in

AUCKLAND FARMERS' FREEZING CO. LTD.

N.Z. CHILLED BEEF JUDGING STANDARDS

"On Hooks" Judging

Works A.A.

Exhibitor _____ Catalogue No. 2

WEIGHT—FORES 284 HINDS 308 TOTAL 592

	SCALE OF POINTS BY MEASUREMENT	Max. Points	M.M.	Points
1	FULLNESS OF MEAT (Eye Muscle Depth)	20	67	17
2	FAT COVER (Depth over Eye Muscle)	15	22	14
3	BLOCKINESS (Length of Leg In Crutch)	20	14¾	17
4	BALANCE OF CARCASE (Fore End—Hind End Wt.)	10	-24	10
5	WEIGHT SUITABILITY	5		4
	SCALE OF POINTS BY EYE JUDGMENT			
6	RIB COVER (Muscle and Fat) 5 and EVENNESS OF FAT DISTRIBUTION 10	15		4 8
7	COLOUR AND TEXTURE OF MUSCLE	5		3
8	COLOUR AND TEXTURE OF FAT	5		4
9	MARBLING OF MUSCLE (Finish)	5		1
42490	TOTAL	100		82

FIG. 7.—Score Card of Carcass of Fig. 6.

permitting the system to be tried out in Competitions, to Mr. J. Anderson of Fletcher's (N.Z.) Ltd., Westfield, and Mr. F. Merritt, Supervising Grader, N.Z. Meat Board, Auckland, for constructive criticism, and to Mr. J. Gibson for the photographic work. The authors are also grateful to Dr. John Hamond, University of Cambridge, and to several panels of Smithfield Market judges for the time and interest applied to examining the efficiency of the method.

REFERENCES

(1) McMEEKAN, C. P., PEIRSON, H. M., JOHNSON, H. E., KNEEBONE, H., MERRIT, F. and ANDERSON, J. D. (1944): *N.Z. J. Sci. and Tech., 26 (Sec. A)*, 157-70.

(2) HIRZEL, R. (1939): *Onders J. Vet. Sci. and Animal Ind., 12,* 379.

THE SEASONAL INCIDENCE OF THE CODLING MOTH (*Cydia Pomonella* L.) IN THE MANAWATU

By Joan Robb, Massey Agricultural College, Palmerston North

Summary

The seasonal incidence of the codling moth in the Manawatu district was observed by trapping moths in liquid bait, trapping migrating caterpillars in trunk bands, and rearing moths from the latter collections.

Observations began in the spring of 1947 after the first moths of the season were on the wing, and were still being made during the second summer (1948-49) at the time of writing this report.

In the first season of observations, it was found that caterpillars migrated from the fruit from 29 December 1947, until 5 May 1948. From cage experiments during this season a partial second generation of moths was indicated.

During the second summer the initial date of the appearance of moths in the field was found to be 21 October 1948. In the first season of observations moths appeared in the field until 24 February 1948.

Introduction

Although the codling moth has attracted much attention the relevant New Zealand literature is limited.

Investigations into the life cycle of the codling moth in New Zealand have been carried out by Dumbleton (1932) in the Nelson district, and by Hamilton (1934) in Canterbury; the former found only one generation per year in Nelson, while the latter demonstrated the occurrence of a partial second generation in Canterbury.

Object of the Investigations

The object of the investigations was to determine the seasonal cycle of the insect, with particular reference to the date of the first appearance of the moth in the spring, the period over which the moths were found, and the number of broods per year.

The knowledge so obtained was intended to provide a basis for timing spraying programmes in the Manawatu district.

Materials and Methods

The moths were trapped in one-gallon fruit tins (Fig. 1), each of which contained a quart of the following mixture, which has been used elsewhere for the same purpose:—

Compressed yeast	3 oz.
Molasses	1½ pt.
Water	19 „

The bait was renewed weekly.

Caterpillar traps consisting of strips of corrugated cardboard three inches wide were wrapped round the trunks of trees a few inches above the ground (Fig. 2). These bands were removed at fortnightly intervals and replaced by fresh material.

The cages which were used for observing the development of the trapped caterpillars consisted of a wooden framework 12 in. x 9 in. covered with mutton cloth (Figs. 3 and 4).

FIG. 1.

FIG. 2.

Fig. 3.

Fig. 4.

Weather Records

In Fig. 5 are plotted data on rainfall, maximum and minimum atmospheric temperatures, hours of sunshine and amount of wind, taken from the records of the Meteorological Station of Grasslands Division, Department of Scientific and Industrial Research, situated about one mile distant from the orchards.

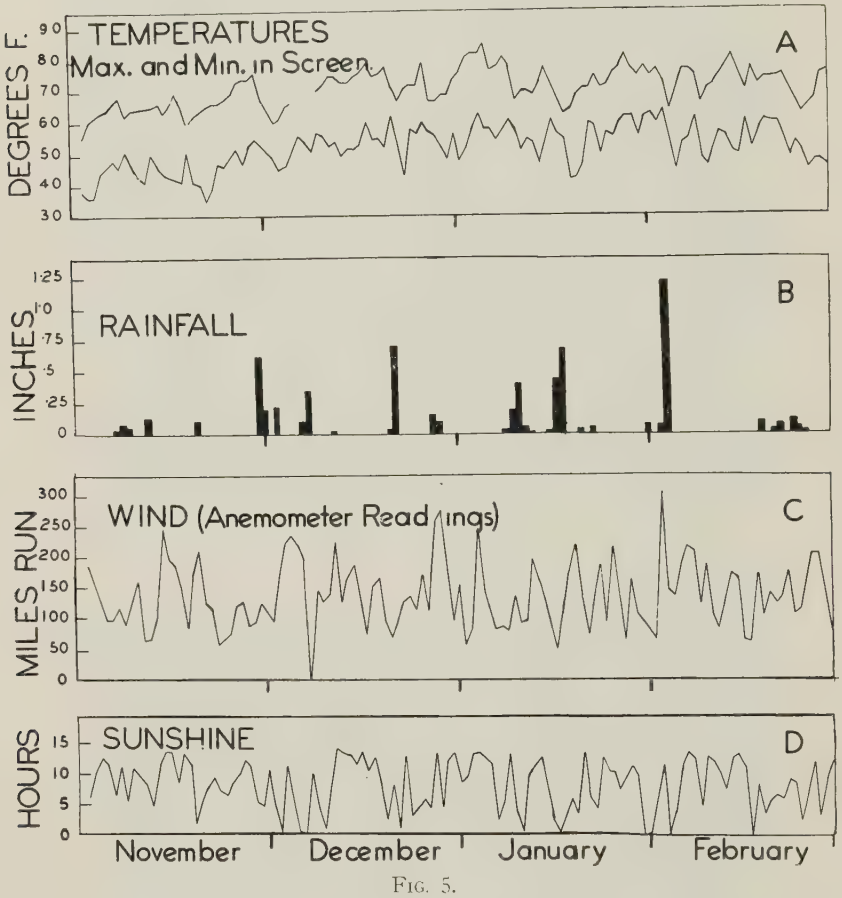

FIG. 5.

Description of the Orchards

The work was carried out in two orchards at Palmerston North: Orchard 1, the Massey College orchard in Albert Street; and Orchard 2, a private orchard.

For the past eighteen years a regular codling moth spray programme had been adopted in Orchard 1, but Orchard 2 had received no insecticidal spray for at least two years immediately prior to the beginning of the present observations.

Orchard 1 contained 368 apple trees of various varieties but only the varieties Cox Orange, Dunn's, Delicious and Rennette du Canada were used in the experimental work.

Between 12 September 1947 and 22 January 1948, Orchard 1 was sprayed eight times with standard mixtures of Bordeaux, Lime Sulphur, Colloidal Sulphur, and Lead Arsenate.

Orchard 2 consisted of 72 trees among which the following varieties were identified: Sturmer, Statesman, Delicious, Golden Delicious, Gravenstein, and Balfour. The apples were allowed to remain on the trees until the caterpillars ceased to be trapped in the bands.

Plan of Work

In order to determine when moths were on the wing, five traps containing the molasses bait were suspended, one per tree, amongst the foliage at heights varying from four to eight feet above the ground. Bi-weekly observations on the numbers of moths trapped began on 30 November 1947, and were continued throughout the summer.

Corrugated cardboard bands were placed on fifty trees in each orchard on 1 December 1947, and fortnightly inspections were made from then until after 5 May 1948.

In order to determine the extent to which a second generation occurred, the caterpillars trapped in the trunk bands were transferred to mutton cloth cages which were set up in the orchards.

These cages were inspected twice weekly and any moths emerging were removed and counted.

No attempt was made to establish the length of the pupal period.

Results

Data on the numbers of moths trapped in the trees are given in Fig. 6 and 7. In Fig. 6 data are presented in the form of numbers of moths caught during weekly periods; and in Fig. 7 data are reassembled in the form of the percentage of the total of the population of moths trapped during the season, found at each inspection date.

The results of the fortnightly catches of caterpillars in the bands are set out in Fig. 8.

Results of cage emergences of the moths from band trappings are contained in Fig. 9.

Discussion

Date of Spring Emergence of Moths

From the results given in Fig. 6, it would appear that the initial date of moth emergence in both orchards in 1947 occurred before 30 November, the date on which observations began. In 1948 observations commenced in time to record the initial emergence of adults and it is seen that in Orchard 1 the first moth was trapped on 21 October, while in Orchard 2 no moths were trapped until 29 October 1948.

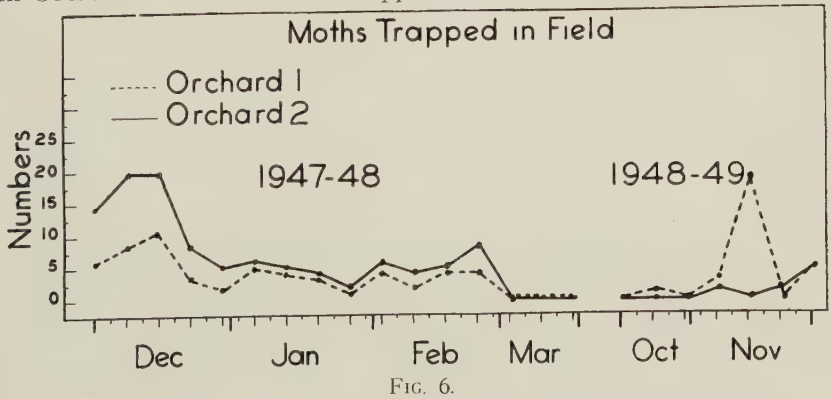

Fig. 6.

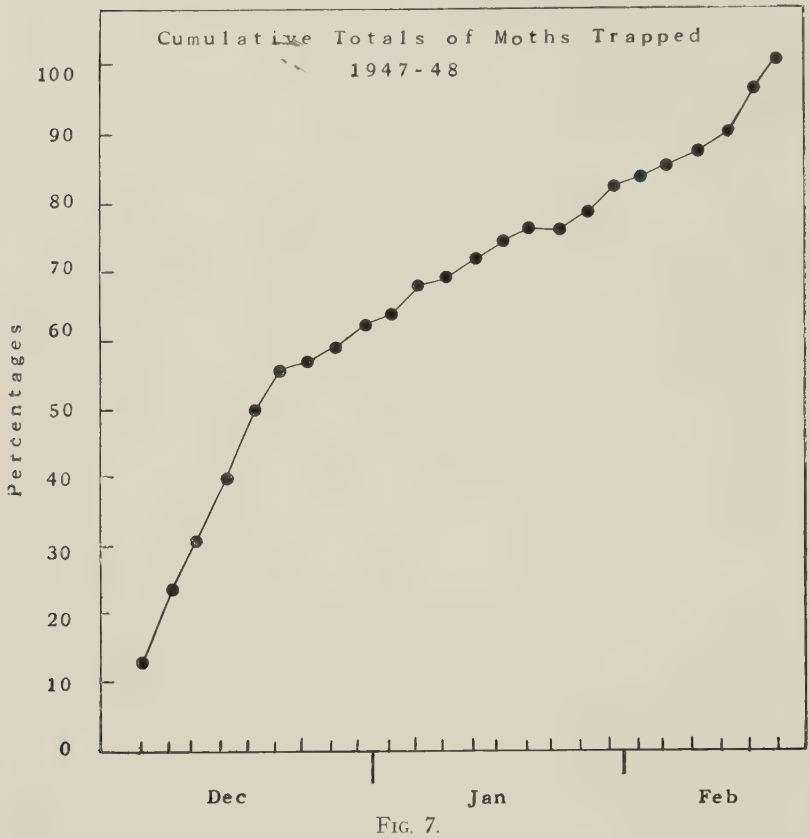

FIG. 7.

Period over which Moths were trapped

From Figs. 6 and 7 it can be seen that moths were trapped intermittently throughout the season from at least 30 November 1947, until 24 February 1948, in the instance of Orchard 1, and to 27 February 1948, in the case of Orchard 2.

The main emergence period of the spring brood as indicated in Figs. 6 and 9, is seen to have extended until about 19 December 1947. From the data obtained early in the summer of 1948-49 it appeared that a similar emergence period was about to be repeated.

Caterpillar Migration

Migrating caterpillars from fruit were first trapped in the bands in both orchards on 29 December 1947. On reference to Fig. 4 it can be seen that in Orchard 2 there was an extended season of considerable caterpillar migration, lasting from the middle of January to the end of April, during which period a peak of numbers was recorded late in March.

As the fruit was picked from the experimental trees in Orchard 1 by 23 March 1948, data for this orchard do not indicate the exact date of the conclusion of the caterpillar migrations. However, in Orchard 2, since the fruit was not picked until the observations had concluded, the final date on which caterpillars were trapped (5 May 1948) may be regarded as the conclusion of the migration of the overwintering caterpillars.

It may be noted that of the total of 1,813 caterpillars trapped during the season, 450 were from Orchard 1, and 1,363 were from Orchard 2. This gives an average of seven caterpillars per tree in the first case and 27.26 caterpillars per tree in the second.

Second Generation of Moths

Although the main populations of moths were trapped in early summer, there was a continued intermittent emergence of moths, which did not clearly indicate the appearance of a second generation. However, the trappings of caterpillars and the cage emergence results (shown in Fig. 9) indicated at least a partial second generation.

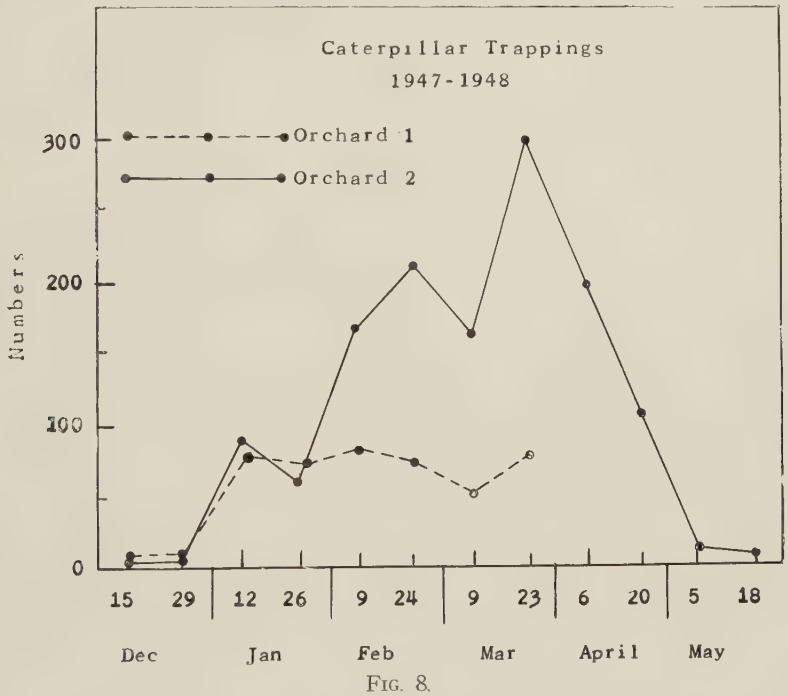

FIG. 8.

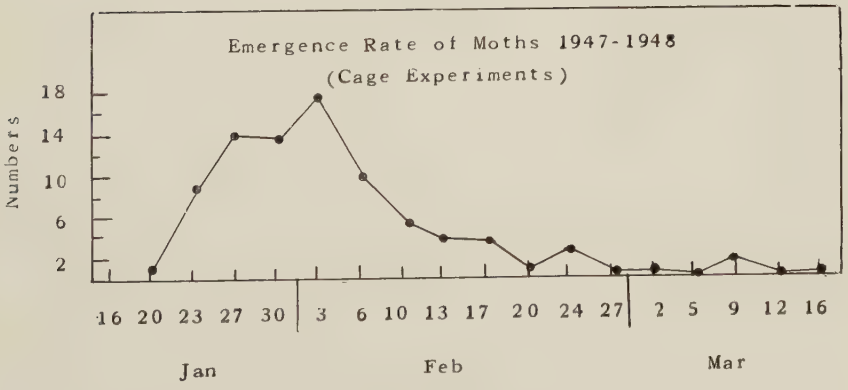

FIG. 9.

From 1,813 caterpillars which were trapped during the 1947-48 season a total of 89 moths emerged the same summer, i.e., 4.36 per cent of the population. These adults emerged over the period from 16 January 1948 to 9 March 1948, and most of them appeared between 23 January and 6 February.

Of the total number of moths which emerged from the cages, 75.3 per cent were from caterpillars which had migrated from fruit during the period 29 December 1947 to 12 January 1948.

A comparison of the number of emergences from cages with the results of field trappings of moths shows that there was no marked correlation during the period from 23 January to 6 February, which has just been mentioned as the main cage emergence period. Although it would be expected that moth emergence under field conditions would extend over a longer period than would be found under cage conditions, it is reasonable to conclude that the intermittent small numbers of moths trapped in the field in the latter part of the season from mid-January, included individuals of the second generation.

Conclusion

In view of the above results, it is suggested that the first spray against the codling moth in the Manawatu be applied early in November. This would allow time for the hatching of the eggs of the first moths, and would provide a poisonous covering on the developing fruit when the young caterpillars begin attacking it. However, as the date of the appearance of the first moths will vary from season to season depending on climatic conditions, it is suggested that each orchardist erect a bait trap, as described in this article, and examine it daily from mid-October onwards. Spray should be applied about 8 to 10 days after a moderate "catch" of moths is secured.

Acknowledgments

The author wishes to express thanks to Mr. J. H. Tetley of the Zoology Department, Massey College, for advice and interest in the project; to Mr. K. C. Hockey of the Horticultural Department, Massey College, and Mr. S. M. Nairn for providing orchard facilities; and to the Horticultural Department, Massey College, for providing financial assistance.

References

Dumbleton, L. J. (1932): *N.Z. J. Sci. and Tech., 14,* 114-7.
Hamilton, A. (1934): *Ibid., 16,* 1-8.

STUDIES IN MONOZYGOTIC CATTLE TWINS.

III. UNIFORMITY TRIALS. MILK AND BUTTERFAT PRODUCTION

By John Hancock, Ruakura Animal Research Station, Department of Agriculture, Hamilton

Summary

1. Possible causes for differences occurring in monozygotic twins living in the same environment are discussed.

2. The results from a uniformity trial on milk production are presented It is concluded that one set of monozygotic twins can, depending on the characteristic in consideration, replace without loss of statistical efficiency two groups of the following numbers of randomly selected animals:

Milk	22
Butterfat	54
Casein	50
Butterfat percentage	15
Casein percentage	10
Casein-butterfat ratio	13
Persistency of lactation	4
Dairy merit	5

3. Herd wastage factors reducing the relative efficiency of monozygotic cattle twins are discussed in terms of the practical use of twins.

Introduction

By virtue of their monozygotic origin, identical cattle twins are theoretically much more valuable for experimental studies than are randomly selected cattle in which hereditary influences always tend to mask the effects of environment. In view of the very wide and important issues at stake in this connection, it is obviously important to establish and measure the superiority of monozygotic twins over ordinary cattle for research purposes. Due to the fairly small number of sets available to him, Bonnier (1) has assumed this superiority and with his material proceeded immediately to studies involving the imposition of experimental treatments. In a later communication Bonnier, Hansson and Düring (2) have estimated that monozygotic twins are at least ten times as efficient as random animals for milk-production studies. However, the data on which this estimate was based was derived from a " split-twin " experiment. As a consequence the productions had to be calculated by converting milk production and growth increments into four per cent milk. Bonnier himself concedes that the value of monozygotic twins thus arrived at can only be regarded as a very rough estimate. The larger number of twins available at Ruakura made it both possible and desirable to establish with some degree of accuracy the relative superiority of monozygotic twins for various experimental purposes.

Factors Causing Differences Within Sets of Monozygotic Twins

Differences occurring within sets of monozygotic twins when the members have been exposed throughout their lives to the same environment may be caused by the following factors:—

Chance

Dahlberg (3) has discussed the rôle chance may play in causing differences within human monozygotic twins. His reasoning is roughly

as follows: If the twins carry a factor which makes them vulnerable to the action of an agency that operates on an average only once in say five years, it may so happen that one member is struck in its first year of life whereas the other may escape unscathed through its whole life. Fertile or infertile matings may be an example of the operation of chance-factors.

Asymetry Reversal

Newman and his co-workers (4) have discussed fully this cause of differences within sets of monozygotic human twins. While he has shown that such differences do occur, there seems to be no direct evidence that any other than rather superficial characteristics are involved. Such characteristics are, for instance, handedness, dentition pattern, and whorls. Newman, however, argues that the great differences in stature, head shape, etc., frequently noted in conjoined twins are due to the asymetry reversal mechanism and sees no reason why such differences may not be caused in ordinary monozygotic twins by the same process. In the Ruakura monozygotic cattle twin material some evidence of mirror imaging has been found, especially in the hair whorls, but whether such twins are less alike in other respects than twins not showing the condition is very doubtful.

The Cleavage Process

Far-reaching differences within sets of monozygotic cattle twins may develop as a result of the cleavage of the embryo from which the twins developed. Cases of gross anatomical defects in one member but absent in the other member of a set of twins have been cited by Hancock (5) as instances of this phenomenon. As it is possible in most cases to exclude sets of this nature, the general usefulness of monozygotic twins need not be impaired.

Uncontrollable Environmental Factors

Pre-natal

According to Newman (4) the differential pre-natal nutrition due to unequal blood supply to the two foetuses, which in twins is caused by anastomosis of the fœtal blood vessels, may result in unequal development in sets of human monozygotic twins. As evidence he cites the fact that at birth, weight differences within sets of monozygotic twins are as great as within dizygotic twins. Newman also believes that the unequal pre-natal nutrition may cause an enduring lowering in the vitality of the affected twins. Ruakura data suggest that there may be pre-natal nutritional factors which cause unequal birth weights in monozygotic cattle twins but that the effect of such factors is not persistent. In the sample of 10 sets of monozygotic twins collected in 1944 the average deviation from mean weights within twins sets was 3.9 per cent at birth but dropped thereafter at a fairly uniform rate to 0.71 per cent at 78 weeks of age.

Hancock (5) has discussed other types of differences present at birth, such as differences in white markings, pigmentation of mouth and skin, whorls, and number of supernumerary teats. As the above characteristics are not likely to be important from a cattle experimental point of view they need not be elaborated in this connection.

Post-natal

The usefulness of monozygotic twins as experimental material need hardly be impaired by within-set differences present already at birth,

as twin sets showing pronounced differences at this stage may be excluded. Uncontrollable environmental factors operating after birth may, however, cause within-set differences which can not always be distinguished from the effects of experimental treatment, thus introducing an element of uncertainty into the interpretation of experimental results.

Under New Zealand grassland farming, dairy cattle are kept on a free food intake system practically from birth onwards. Under these conditions it is possible that not easily controllable environmental factors play a considerable part in causing differences within sets of monozygotic twins. Such factors are, for instance, qualitative and quantitative differences in feed intake and varying intensities of bacterial or parasite infestation.

In view of these considerations it was decided at Ruakura to employ a number of unselected monozygotic twin sets in "uniformity" trials. Briefly, the procedure followed in these trials was to measure the performance of monozygotic twins which had been treated alike from birth onwards. The degree of similarity in various characteristics shown by monozygotic twins under such conditions, largely determines their usefulness as experimental animals. It must be noted that the effect of uncontrollable factors may vary from character to character. It is, therefore, important to determine the relative uniformity of twins in respect of all characters in which the experimentalist is interested.

In the present paper the result from uniformity trials on milk production only are reported. Subsequent papers will cover other important aspects.

Experimental Animals

Nine sets of monozygotic twin heifers calving for the first time were included in this study. The animals were brought to Ruakura at an early age, whereafter they were run as one herd. They were fed on whole milk till they were three weeks old. In the following three weeks they were gradually changed to skim-milk. The milk was given at a daily rate of 12 per cent body weight up to five months of age, when weaning took place. The animals were provided with the best pastures available throughout their growth period. When pasture supply was inadequate in slow growing periods, they were given either hay or silage or both *ad lib.* to supplement the grass available. Each individual was thus given the opportunity to develop according to its inherited capacity within the limits set by a relatively optimal pastoral environment. No attempt was made to equalize "within" or "between" set differences which occurred in the growth of the twins.

The brand numbers and breed-type of the experimental animals are shown in Table I.

It will be noted that out of the nine sets one was pure-bred Jersey and the remainder grade Jerseys. Of the latter only four sets were sired by pedigree Jersey bulls. Five sets showed at least some traits of either Ayrshire, Shorthorn, or Hereford.

When monozygotic twins are acquired by Ruakura Animal Research Station, no consideration is taken of the breed or productivity of their parents. The animals included in this trial can, therefore, be considered a small random sample of the cattle population of the South Auckland land district, the main dairying area of New Zealand.

TABLE I. BREED TYPE OF EXPERIMENTAL ANIMALS

Twin No.	Breed type of twin according to appearance.	Breed of Parents according to Breeder.	
		Sire.	Dam.
T1 - T2	Medium Grade Jersey	Grade Jersey	Grade Jersey
T3 - T4	Medium Grade Jersey	Pedigree Jersey	Grade Jersey
T11 - T12	Jersey × Ayrshire	Pedigree Jersey	Ayrshire × Jersey
T13 - T14	Medium Grade Jersey (Shorthorn traits)	Pedigree Jersey	Grade Jersey
T17 - T18	Medium Grade Jersey (Shorthorn traits)	Grade Jersey	Grade Jersey
T23 - T24	Pure Bred Jersey	Pedigree Jersey	Pedigree Jersey
T27 - T28	Medium Grade Jersey	Grade Jersey	Grade Jersey
T29 - T30	Medium Grade Jersey	Grade Jersey	Grade Jersey
T31 - T32	Low Grade Jersey (Hereford trait)	Grade Jersey	Grade Jersey × ¼ Hereford

Mating and Calving

The birth dates, calving and subsequent conception dates, and body weights of the experimental animals three weeks after calving are shown in Table II.

TABLE II. BIRTH, CALVING AND CONCEPTION DATES, AND BODY WEIGHTS OF EXPERIMENTAL ANIMALS THREE WEEKS AFTER CALVING

Twin No.	Birth Date.	Calving Date.	Conception Date.	Weight lb.
T 1	10/8/44	14/8/46	25/10/46	691
T 2		17/8/46	3/11/46	690
T 3	9/8/44	18/9/46	25/11/46	596
T 4		20/8/46	Empty	638
T11	28/8/44	19/8/46	31/12/46	626
T12		17/9/46	12/11/46	638
T13	22/7/44	20/8/46	31/10/46	694
T14		15/8/46	20/10/46	711
T17	6/7/44	27/8/46	6/11/46	813
T18		14/8/46	31/10/46	834
T23	20/7/44	16/8/46	1/11/46	658
T24		3/9/46	12/11/46	637
T27	25/9/44	4/9/46	31/10/46	547
T28		24/9/46	Empty	596
T29	13/7/44	17/9/46	13/11/46	704
T30		5/8/46	24/10/46	690
T31	29/1/45	17/9/46	Empty	606
T32		16/9/46	26/12/46	578

Because of the intra-seasonal variations in food supply, which cannot be entirely equalized for heifers calving at different dates and ages, it would have been ideal to have all animals calving at the same date and at the same age. This was not possible for several reasons:—

(1) The birth dates of the twins were spaced over a fairly wide time-period.

(2) Oestrus occurred at random over approximately 21-day periods. This applied to heifers of the same set as well as to heifers of different sets.

(3) A 100 per cent conception rate, could not be obtained.

In order to equalize, to some extent, the differences in environment due to age at calving, an attempt was made to calve down the younger heifers at later date but at a lower age than the older ones. At the same time it was attempted to restrict the within-set differences in calving dates to a minimum.

Table II shows that the range in calving dates was 43 days and the age at calving varied from 85 to 114 weeks. The within-set differences in calving dates varied from 1 day to 43 days. A statistical analysis showed that the between-set differences in calving dates were not significantly greater than the within-set differences, but the age differences at calving were significantly (strongly) greater between sets than within sets. From Table II and Fig. 1, it can be seen, however, that the greater between-set differences in age at calving were mainly due to the fact that two animals (T31 and T32) belonging to the same set calved simultaneously at an appreciably lower age than the others. This set contributed thus materially to the between-set variance without increasing at all the within-set variance. For reasons which will be discussed later, the production data of T31 - T32 are not included in the final computations on which the main conclusions of this work are based. If they are, therefore, also excluded from the analysis of calving dates and age at calving, it is now found that the within-set differences in calving dates were greater, although not significantly so, than the between-set differences, and that within-set age differences at calving are not significantly greater than between sets. It may, therefore, be concluded that if production results were influenced by these two factors, members of the same sets were affected to the same extent as animals of different sets.

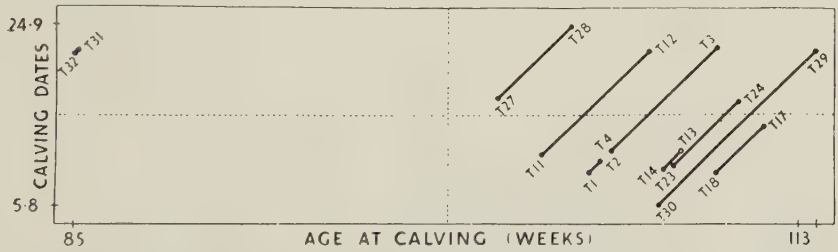

Fig. 1.—Diagram showing age at calving and calving dates of the experimental animals.

Feeding

The animals were pasture fed throughout the experimental period. At times of grass-shortage supplementary fodder, mainly in form of silage and roots, were given. Hay was fed in the spring in small quantities in order to correct the laxative properties of the pasture at this time of the year. Fig. 2 shows the amounts of supplements given

per day per cow. It will be noted that the supplements probably at no time amounted to more than a quarter of the daily dry matter intake.

Method of Collecting Data

The cows were milked twice a day and the milk weighed at each milking. Butterfat percentage was measured daily by the Gerber method on a composite sample from the two milkings. Casein was estimated daily according to the modified Walker method as described by McDowall (6). The cows were weighed once a week.

RESULTS

The productions have been expressed in terms of a 252-days lactation period. After this time, differences in state and stage of pregnancy are likely to affect the productions markedly. As can be seen from Table II three animals belonging to three different sets did not hold to service and remained empty throughout their lactations.

Table III shows the total milk, butterfat, and casein productions, and the butterfat and casein percentages of the nine sets.

TABLE III. MILK BUTTERFAT AND CASEIN PRODUCTION OVER 252 DAY LACTATION PERIOD

Twin No.	Milk.		Butterfat.		Butterfat.		Casein.		Casein.	
	lb.	Diff.	lb.	Diff.	%	Diff.	lb.	Diff.	%	Diff.
T 1	5042	233	266	14	5·28	0·03	141	3	2·79	0·06
T 2	5275		280		5·31		144		2·73	
T 3	4032	125	240	8	5·95	0·02	103	7	2·59	0·04
T 4	4157		248		5·97		110		2·63	
T11	3833	212	222	6	5·78	0·07	99	6	2·59	0·01
T12	3621		216		5·85		93		2·58	
T13	3943	142	212	1	5·37	0·21	98	0	2·48	0·07
T14	4085		211		5·16		98		2·41	
T17	5781	258	323	8	5·58	0·03	154	5	2·66	0·01
T18	5623		315		5·61		149		2·65	
T23	200	73	11	6	—	—	7	3	—	—
T24	127		5		—		4		—	
T27	4120	20	216	5	5·25	0·13	109	0	2·64	0·02
T28	4100		221		5·38		109		2·66	
T29	5205	490	242	3	4·65	0·35	129	6	2·47	0·09
T30	5695		245		4·30		135		2·38	
T31	349	14	17	0	—	—	9	0	—	—
T32	363		17		—		9		—	

Two facts are immediately evident—

(1) The productions of the members of the same sets were very similar.

(2) There were great differences in productive ability between sets.

The average within-set differences were 166 lb., 5.6 lb., and 3.3 lb., for total milk, butterfat, and casein, respectively, while the difference between the lowest and highest producing sets were 5,508 lb. milk, 311 lb. butterfat, and 146 lb. casein. The production range remains high even if the two sets of very poor producers (T23 - T24 and T31 - T32) are disregarded, i.e. 1,955 lb. milk, 107 lb. butterfat, and 54 lb. casein. Excluding the two above mentioned sets the average within-set differences in

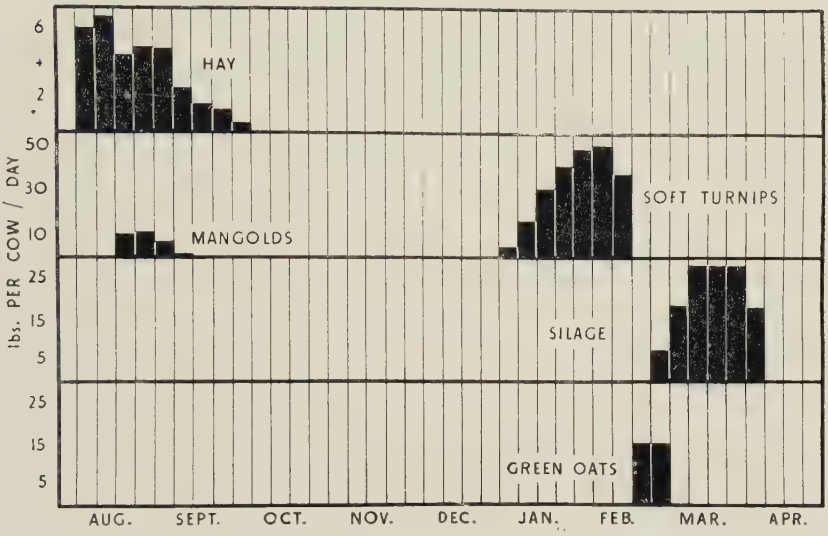

Fig. 2.—Supplementary feeding.

butterfat and casein tests amounted to 0.13 per cent and 0.04 per cent respectively, whereas the range between sets was 1.5 per cent and 0.33 per cent. It can be noted that if the seven sets which produced normally were divided into two groups, one to include the "odd" and the other to include the "even" members of each set, the average difference between the two groups would only have amounted to 88 lb. of milk and 2 lb. of butterfat.

The variations in monthly (28-day periods) milk, butterfat, and casein productions are shown in Fig. 3, and the variation in butterfat percentage, casein percentage, and the casein-butterfat ratio are shown in Fig. 4. It can be noted that within-set differences in these characteristics were small during most periods except for the two sets T3 - T4 and T29 - T30. In the beginning of the lactation, T4 produced more milk and butterfat than T3. Towards the end, however, this situation tended to be reversed with the consequence that the total milk and butterfat production of this set was fairly similar. The set T29 - T30 behaved somewhat differently, T29 giving appreciably smaller milk yield than T30, mainly due to low production in the first and seventh periods. The total butterfat productions of these twins, however, were very similar due to the consistently higher butterfat percentage of T29. On the whole there seemed to be, within sets, a tendency to compensate for a lower milk production by a higher butterfat percentage.

Between-set differences and within-set similarities in the shape of lactation curves are notable as, for instance, in the case of the two sets T11 - T12 and T13 - T14 which yielded essentially similar total productions of milk, butterfat, and casein. T11 - T12 began the lactation with a very low production. They did not reach their maximum milk yield until the seventh period, their maximum butterfat yield until the eighth (T11) and ninth (T12) periods, and their maximum casein yield until the sixth (T12) and seventh (T11) periods. In contrast both T13 and T14 reached their peak period very early in the lactation, i.e. in the second, third, and first period for milk, butterfat, and casein respectively.

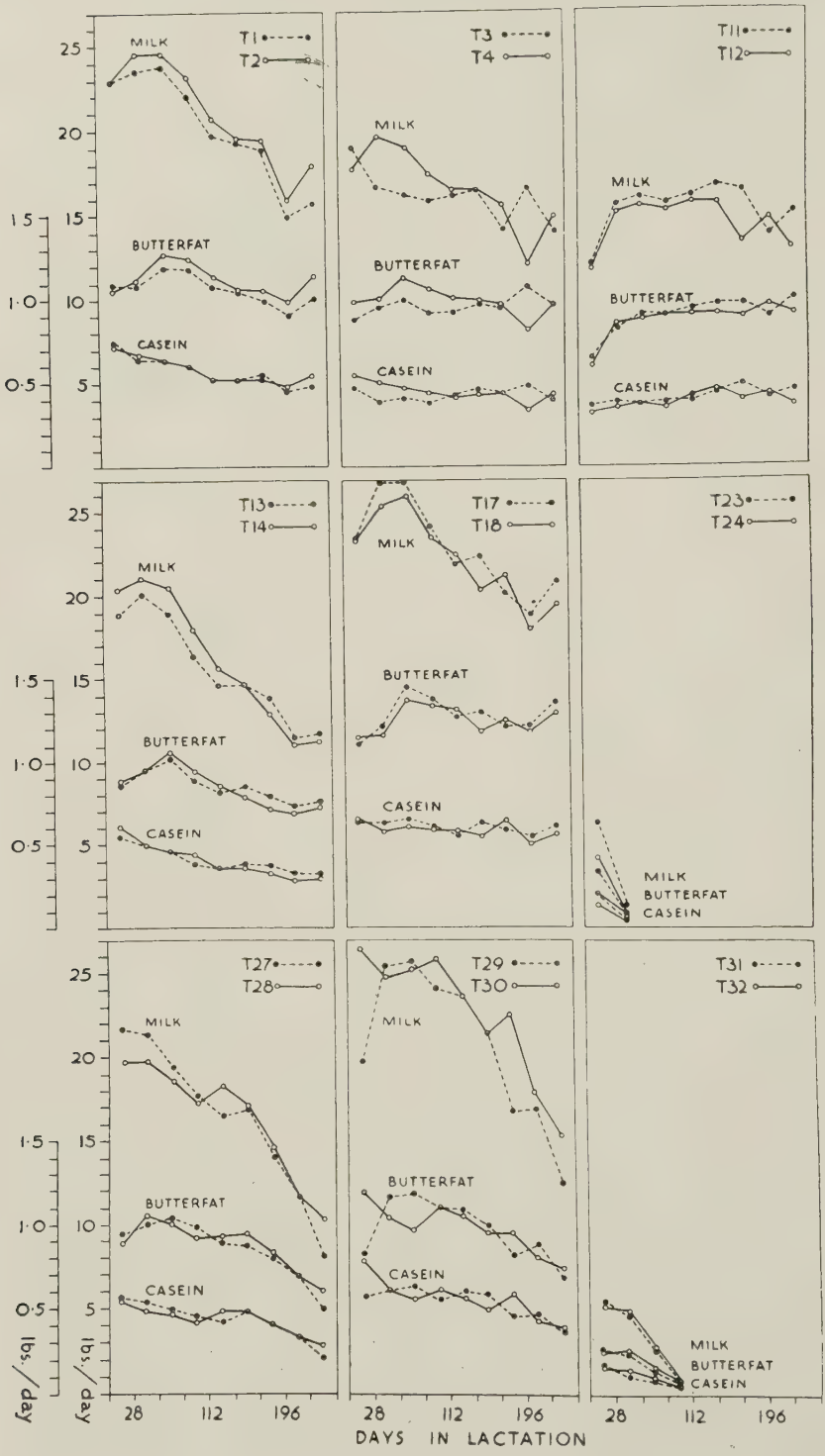

FIG. 3.—Variations in the milk, butterfat and casein production.

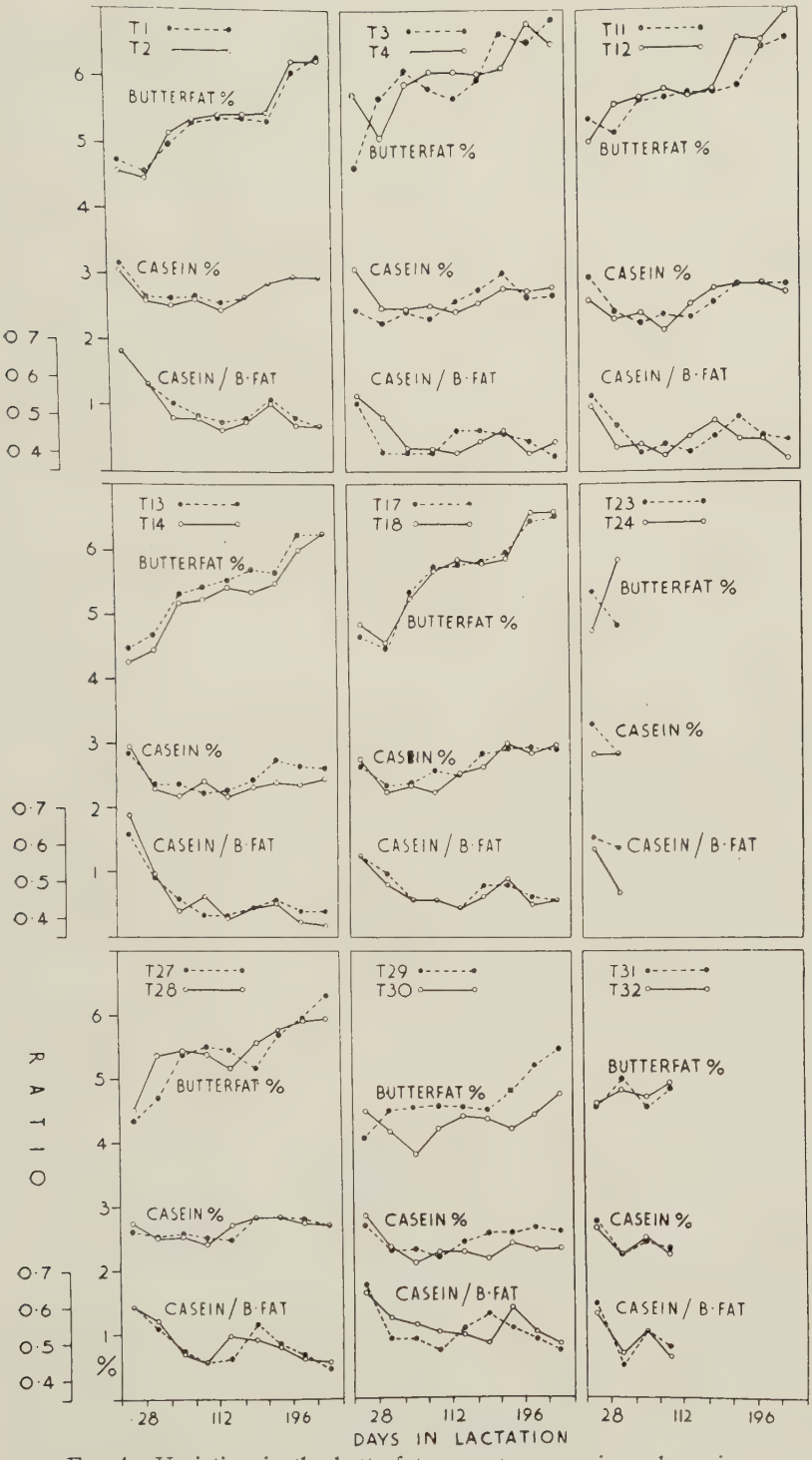

FIG. 4.—Variation in the butterfat percentage, casein and casein-butter ratios.

No specific environmental factors were observed which could account for the extremely low yields of the sets T23 - T24 and T31 - T32. The fact that the within-set performances of the animals were so similar seems to indicate congenital deficiencies. From the point of view of breeding quality, the sets were rather different: T23 - T24 were pure bred Jerseys sired by a bull of "Merit" class (as defined by the N.Z. Dairy Board) (7) whereas T31 - T32 were of mixed ancestry showing unmistakable Hereford traits ("brockle-face" colour pattern and long narrow udders). It is quite possible that the intrinsic physiological background of the low yields was not the same in both cases. T23 - T24 possessed quite large and well formed udders which, however, had a "fleshy" quality. Petersen (8) associates udders of this type with animals which remain poor producers presumably because, although otherwise normal, they lack the oxytocic principle which governs the milk let-down process. The consistency of the udders and the very small udder size of T31 - T32 suggested, on the other hand, that the low yields of these animals were due to inadequate development of secretory tissue. Their udders were slightly distended before milking, becoming absolutely slack afterwards. As these two sets obviously were sub-normal and their inclusion exaggerates the relative value of twins, they have been omitted from the discussion in the remainder of this paper.

With the data in Table III as basis, following characteristics of the twins have been calculated:

(1) Dairy merit.
(2) Lactational persistency.
(3) Casein/Butterfat ratio.

"Dairy merit" or "Lactational drive" has been defined by Brody (9) as: "the biological efficiency of milk production as measured by the percentage of consumed TDN (Total digestible nutrients) energy which is converted into milk (FCM) (Four per cent corrected milk) energy.

TABLE IV. DAIRY MERIT, PERSISTENCY OF LACTATION AND CASEIN-BUTTERFAT RATIO

Twin No.	Dairy Merit.		Persistency of Lactation.		Casein/Butterfat Ratio.	
	Index.	Within-set Diff.	Index.	Within-set Diff.	Index.	Within-set Diff.
T 1	34·5	1·8	0·95	0·01	0·53	0·02
T 2	36·3		0·96		0·51	
T 3	34·7	1·9	1·00	0·05	0·44	0·00
T 4	32·8		0·95		0·44	
T11	30·8	1·6	1·04	0·02	0·45	0·02
T12	29·2		1·02		0·43	
T13	27·2	0·4	0·90	0·02	0·46	0·01
T14	26·8		0·88		0·47	
T17	34·9	1·7	0·98	0·02	0·48	0·01
T18	33·2		1·00		0·47	
T27	35·5	2·5	0·86	0·02	0·50	0·01
T28	33·0		0·88		0·49	
T29	32·2	2·0	0·88	0·07	0·53	0·02
T30	34·2		0·95		0·55	

As it was not possible to obtain the TDN consumption, indirect dairy merit indices were calculated according to following slightly modified formula derived by Gaines (10):

$$\frac{1000\ (FCM)}{W}$$

where FCM = Average daily production in lb. of four per cent milk for a partial lactation of nine 28-day periods.

W = body weight at three weeks after calving.

The results are shown in the first column of Table IV.

It will be seen that the twins show fairly good concordance with an average within-set difference of 1.7 points as compared with a range of 8.4 points between the highest and the lowest scoring sets.

Persistency of lactation was measured by the use of the following formula devised by Ludwick and Peterson (11):

$$P = \frac{\frac{X_{2n}}{X_1} + \frac{X_{3(n-1)}}{X_2} + \frac{X_{4(n-2)}}{X_3} + \frac{X_{n\,(n-(n-2))}}{X_{(n-1)}}}{n\ (n-1) - (n-1)\ \frac{(n-2)}{(2)}}$$

where P = Persistency index.

X = (with the aid of subscripts) designates the production of any particular period.

N = the number of divisions into which the lactation period is divided.

For the present purpose the butterfat productions were used. The first 28 days were omitted and the remainder of the 252-day lactation was divided into four equal periods. The indices thus calculated are shown in the second column of Table IV. Here again the similarities within sets of twins are evident. The average within-set differences were 0.03 points compared with a range of 0.16 between the highest and lowest scoring sets.

The ratio of casein to butterfat in milk is of importance in the evaluation of milk quality, especially in cheese manufacture. The ratios calculated are shown in the last column of Table IV. It will be seen that the within-set differences were very small, averaging only 0.013, when compared with the range of 0.10 between two sets showing highest and lowest average ratios.

Relative Value of Twins in Production Experiments

In the absence of environmental interactions the usefulness of monozygotic twins as material in production experiments depends on the relationship between the following two factors:—

(a) Differences in production occurring within sets of twins kept under uniform conditions.

(b) Differences in production occurring between unrelated animals kept in the same conditions.

Both factors can be calculated from the data presented in Table III, (a) as the within-set variance and (b) as the between-set variance. The number of animals chosen at random which one twin may replace without loss of statistical efficiency can be computed, as shown by Dick and Whittle (12) as the ratio:—

$$\frac{\text{between-set variance component}}{\text{within-set variance component}}$$

or as—

$$\frac{\dfrac{(\text{between-set variance})}{(\text{within-set variance})} - 1}{2}$$

In the following, the values thus computed will be known as the "twin efficiency values."

Due to the relatively small sample of twins used both variances may have fairly large errors. The computed efficiency values must, therefore, not be regarded as universal constants but only as guides to the relative usefulness of monozygotic twins in production trials.

The two variances and the twin efficiency values for milk, butterfat, casein, butterfat percentage, and casein percentage, are presented in the first three columns of Table V. For reasons already given the two sets T23 - T24 and T31 - T32 have not been included in these calculations.

TABLE V. TWIN EFFICIENCY VALUES

Characteristic.	Between Set Variance.	Within Set Variance.	Twin Efficiency Value.	Variance Between Unrelated Animals.	Twin Efficiency Value.
Milk	1,283,036	28,604	22	886,548	31
Butterfat	2,916	27	54	2,733	101
Casein	987	9·7	50	—	—
Butterfat percentage	0·49	0·016	15	0·20	13
Casein percentage	0·029	0·0013	10	—	—

The twin efficiency values for butterfat and casein production are very high, viz., 54 and 50 respectively, indicating a marked superiority of monozygotic twins over heifers selected at random for experiments on these characteristics. Although the efficiency values for milk, butterfat percentage, and casein percentage, are lower, viz. 22, 15, and 10, respectively, they still seem sufficiently high to warrant the exclusive use of monozygotic twins in most trials involving the measurement of the effects of various environmental factors on the above characteristics.

Bonnier (2) has shown that a rough check on twin efficiency values can be obtained by calculating the ratio:—

$$\frac{\text{variance between unrelated animals}}{\text{variance within-sets of monozygotic twins}}$$

Lactation records of twelve 2-year-old heifers in another herd at Ruakura Animal Research Station were available for the calculation of this ratio. The fact that these animals were of the same age, calved at the same time and were reared and milked under the same conditions as the twins seems to validate the test. The variances occurring in this sample for milk and butterfat productions and for butterfat test, calculated on the basis of a 252-days lactation period, are shown in the fourth column of Table V. The twin efficiency values computed from these variances and the within-set variances obtained in the twin uniformity trial are shown in the last column of the same table.

It will be noted that the values thus computed for milk and butterfat production are one and a half times and twice respectively as great as the previous ones.

This situation is either a consequence of the higher co-efficients of variation obtaining for milk and butterfat yields in the herd of non-twins compared with those in the twin herd, or of the high sampling fluctuations of the estimates of variance derived from such a small sample.

The efficiency value for butterfat percentage remains almost unchanged, thus reflecting the similarity of the coefficients of variation for this characteristic in the two samples of heifers. No data equivalent to the milk and butterfat yields were available for casein yield.

Finally it must be remembered that the variation between heifers selected at random may be greater than that between twin pairs. Even so, these data would indicate that if the within-set variance were twice as great as in the present sample of monozygotic twins, then the efficiency for butterfat production would still remain at approximately 50.

The twin efficiency values for Dairy Merit, persistency of lactation, and casein-butterfat ratio, are shown in Table VI together with the between-set and within-set variances from which they were derived.

Table VI. Twin Efficiency Values

Characteristic.	Between Set Variance.	Within Set Variance.	Twin Efficiency Value.	Variance Between Unrelated and Non-twins.	Twin Efficiency Value.
Dairy Merit	17·5	1·6	5	32·9	20
Lactational Persistency	0·0064	0·0008	4	0·0018	2
Casein-butterfat Ratio	0·0029	·00011	13		

As the Dairy Merit index is a function of FCM production and body weight, and as FCM productions show a high twin efficiency value of 44, it is obvious that the relatively low value found for the Dairy Merit index is due to the influence of the body weight factor. As can be seen from the weight figures given in the last column of Table II, this factor operates in two ways:—

(1) The better producing sets tended to be heavier;

(2) Within-set differences in body weights were not reflected in production differences in favour of the heavier twin.

As a consequence of (1) above, the between-set variance for Dairy Merit became relatively small while as a consequence of (2) the within-set variance was relatively large. The net result of this situation was a low efficiency value for Dairy Merit.

In this connection it can be pointed out that body weight changes of some amplitude occurred within several sets of twins throughout their lactation. (See Fig. 5.) As these changes were not accompanied by differences in production it can be concluded that butterfat production tended to be maintained in spite of uncontrollable environmental factors which were sufficiently strong to cause appreciable fluctations in body weights within sets.

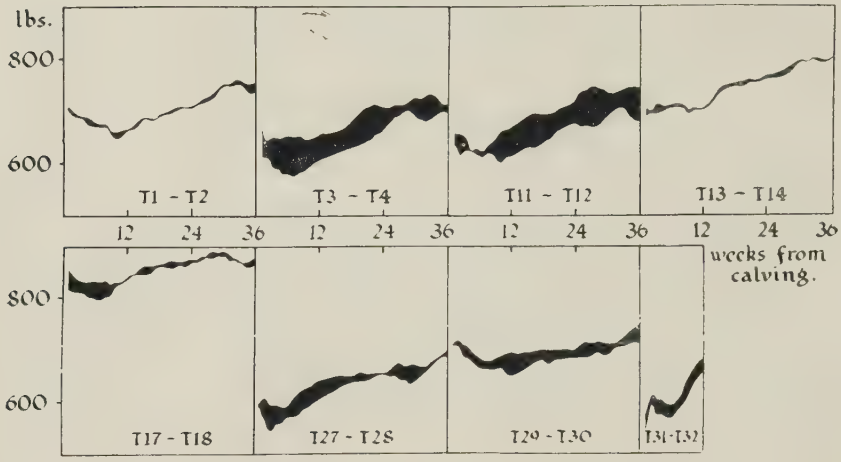

FIG. 5.—Body weight changes of the experimental animals. The black areas indicate within-set differences throughout lactation. Because of the short time they were in milk T23 - T24 are omitted. Growth changes for T31 - T32 are shown only for the time they were in production.

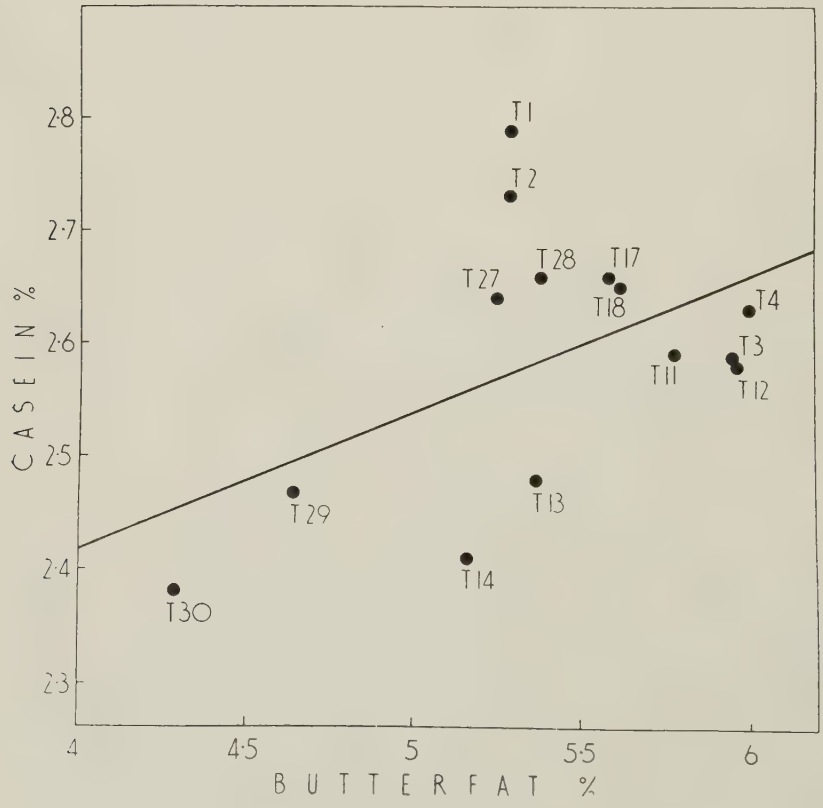

FIG. 6.—Relationship between casein percentage and butterfat percentage.

The efficiency value for Dairy Merit increases to 20 if the variance observed in the sample of non-twins, previously described, is used for computation instead of the between-set variance. This is a reflection of the much higher degree of variation occurring in the non-twin sample. The efficiency value for persistency of lactation was only 3 and decreased to 2 by the use of the non-twin variance.

For the casein-butterfat ratio the twin-efficiency value, viz. 13, is of approximately the same magnitude as for the butterfat and casein percentages. In Fig. 6 the casein percentages have been plotted against the butterfat percentages and the regression line (calculated by the method of least squares) has been drawn. Two facts stand out:—(1) there is a wide scatter of the points round the line, (2) the points for the numbers of each set of twins lie very close together. It thus seems that the similarity in the twin efficiency values between the casein-butterfat ratio on the one hand and the casein and butterfat percentages on the other hand, does not depend on a general high correlation between the two latter characteristics. That the twins, in spite of this low correlation, show a high concordance in casein-butterfat ratio indicates that the casein and butterfat percentages are the expressions of at least partly independent genetical factors. Bonnier and Hansson (13) have reached a similar conclusion as a result of a more searching study.

The relatively high efficiency value obtained shows that monozygotic twins are very useful as material for trials in which the aim is to measure environmental effects upon the casein-butterfat ratio. That some such factors exert a marked influence is generally known, but their exact nature and relative importance are obscure. The data obtained in the present trial illustrates a case in point. From Fig. 4 it will be seen that the monthly values for casein-butterfat ratio show a similar trend for all animals (except perhaps for T13 - T14) with peaks in the beginning and approximately in the middle of the lactation. The second peak must wholly or partly be due to unknown environmental factors for the reason that the members of those twin sets (T3 - T4, T11 - T12, T27 - T28, and T29 - T30) which calved relatively far apart, exhibited this peak at different stages of their lactation.

The between-set and within-set coefficients of variation show more clearly than the respective variances the reasons for the differences in efficiency values obtained for the eight characteristics discussed. They are, therefore, shown in Table VII (in order of decreasing efficiency values).

Table VII. Co-efficients of Variation

Characteristics.	Twin Efficiency Value.	Between-set Co-eff. of Variation.	Within-set Co-eff. of Variation.
Butterfat yield	54	10·9	1·1
Casein yield	50	13·0	1·4
Milk yield	22	12·0	1·9
Butterfat percentage	15	6·5	1·3
Casein-butterfat ratio	13	0·6	0·1
Casein percentage	10	3·3	0·8
Dairy Merit Index	5	6·5	2·0
Persistency of lactation index	4	4·2	1·5

It will be seen that the high efficiency values for butterfat and casein yields relative to that obtained from milk production are mainly due to smaller within-set variances. The smaller efficiency values for the other five characteristics are mainly due to small between-set variances accompanied in some cases by fairly large within-set variances.

Discussion

From a practical point of view the usefulness of monozygotic twins as experimental material depends obviously on other factors than the efficiency values alone. These factors are in general connected with the problems of wastage. If one member of a set of twins dies or becomes useless for experimental purposes, it is obvious that in most cases the co-twin must also be excluded. The wastage in twin material tends, therefore, to be twice as high as in ordinary cows.

Experience gained at Ruakura indicates that the main causes of wastage amongst the twins are as follows:—

(1) Within-set differences in time of calving;
(2) Barrenness;
(3) Disease;
(4) Accidents; and
(5) Deaths.

Difference in time of calving is probably the most serious factor. Monozygotic twins do not tend to come on heat or become pregnant upon service simultaneously. Wide discrepancies in calving time (and age at calving) may consequently occur. In such cases seasonal variations in feed supply may cause production differences which cannot be separated from the effects of experimental treatments. Ruakura data suggest that in order to ensure reasonable precision in the experimental results no greater than a forty-days interval between the calving dates of a set of twins should be allowed.

Temporary sterility may result in one twin remaining empty after its co-twin has conceived. Attempts have been made at Ruakura to bring twin sets of this type back "into step" by drying off the fertile member immediately after calving and by mating both animals thereafter within the shortest possible space of time. Data on the subsequent performance of twins treated in this way are being collected.

White scours, mastitis, and abortion, are the three most common diseases encountered in the work at Ruakura. As all these diseases seem to affect monozygotic twins as if they were random animals, they may cause extra-experimental differences in production. However, in the case of white scours, it has been noted that a calf after recovery generally reaches rapidly the weight of its co-twin. It is, therefore, unlikely that the usefulness of a twin set thus affected is impaired for the purpose of production trials. Experience at Ruakura has shown that wastage due to mastitis may be almost entirely avoided through prompt treatment with penicillin. Abortion in a twin naturally spoils the set at least for one lactation. In this connection, it may be observed that many sets of twins which have been affected through causes listed above may, nevertheless, incidentally provide useful information on important subjects. The influence on production of such factors as, for instance, calving age and time, abortion, accidental loss of one or two mammary glands, can be measured conveniently in this manner. Hansson (14)

(15) has already shown what can be achieved by the proper use of otherwise useless twin material. Since all of these factors affect also non-twin cattle, the results indicate that monozygotic twins have considerably greater value than non-twins to the experimentalist seeking to measure production effects.

Acknowledgments

The author wishes to acknowledge his great indebtedness to Mr. B. Quigley, in whose care the experimental animals remained throughout the trial and who, together with Mrs. N. Roach, did much of the statistical computations. Thanks are also due to Mrs. A. D. Clarke who carried out the butterfat and casein tests. Finally, it is the author's pleasant duty to thank Dr. C. P. McMeekan, Superintendent of Ruakura Animal Research Station, for encouragement and helpful criticism.

(1) Bonnier Gert (1946): *Acta Agr. Suecana 1,* 139-46.

(2) Bonnier Gert, Hansson Artur and During Troed (1946): *Kungl. Lantbruks—Akademiens Tdskr LXXXV, 5,* 455-67.

(3) Dahlberg Gunnar (1945): *Nature, 156,* 539.

(4) Newman Horatio N., Freeman Frank N. and Holzinger Karl Y. (1937): Twins: A study of Heredity and Environment. Second Impression. The University of Chicago Press, Chicago, Illinois.

(5) Hancock John (1949): Studies in Monozygotic Twins: II. Recognition of Monozygotic Cattle Twins. *N.Z. J. Sci. and Tech., 31* (*Sec. A*) (2), 1-41.

(6) McDowall, F. H. (1935): The Walker test for Casein in Milk. *Dairy Res. Inst.* (*N.Z.*), Publ. No. 68.

(7) Sire Survey and Merit Register (1949): Herd Recording Council, N.Z. Dairy Board, Fifth Edition, Wellington.

(8) Petersen, W. F. (1942): Agr. Expt. Stn., University of Minnesota. Bull. 361.

(9) Brody, Samuel (1945): Bioenergetics and Growth. Reinhold Publishing Corp., New York, U.S.A.

(10) Gaines, W. L. (1946): *J. Dairy Sci., XXIX,* 259-72.

(11) Ludwick, T. M. and Peterson, W. E. (1943): *J. Dairy Sci., XXVI,* 439-45.

(12) Dick, I. D. and Whittle, P. (1949): Personal communication.

(13) Bonnier Gert. and Hansson Artur (1946): *Acta Agr. Suecana 11,* 171-84.

(14) Hansson Artur (1946): *Acta Agr. Suecana 1,* 154-62.

(15) —— (1948): *Acta Agr. Suecana 111,* 1, 71-4.

CONTROL OF BACTERIAL-SPOT OF PLUM

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Summary

Experiments commenced in 1942 on control of bacterial-spot on plums by spray treatments were continued during the four seasons 1944-45 to 1947-48.

Bordeaux, lime sulphur and Fermate (ferric dimethyl-dithio-carbamate) were tested, the first only giving significant control.

Results suggested that control was improved by increasing the concentration of Bordeaux and by increasing the number of applications.

Four applications of Bordeaux during fruiting season reduced fruit infection from 38.9 per cent to 6.8 per cent in one season and from 19.6 per cent to 5.5 per cent in another.

None of the spray materials caused fruit injury, but both Bordeaux and lime sulphur damaged the foliage, damage being most severe in the early part of the season. When the first two applications were made with Bordeaux 2-4-100 (2 lb. copper sulphate, 4 lb. hydrated lime, 100 gal. water) and the second two with Bordeaux 3-6-100, foliage damage was not serious and satisfactory control was secured.

Introduction

In a previous paper (Reid, 1945) it was shown that fruit infection of plums by bacterial-spot could be reduced by applying Bordeaux sprays to infected trees. Since then further trials in the same orchard have been carried out to check this result and to determine the number of applications and optimum concentrations required. The trees were heavily infected on buds, twigs, laterals and leaders and a satisfactory method of eliminating the disease in trees is not known. On the other hand protection of fruit against infection is possible and trials described here were concerned with treatments for this purpose only. The block consisted of 75 George Wilson and 62 Doris trees approximately eight years old, including those used in the previous trial (Reid, 1945).

Experimental Methods

In each season a Bordeaux spray 8-8-100 for control of bladder-plum (*Taphrina pruni*) and shot-hole (*Phyllosticta prunicola*) was applied at bud-movement as part of standard orchard practice. In the autumn an additional Bordeaux 6-8-100 spray was given to limit bacterial infection of autumn growth. It is probable that these sprays reduced the amount of disease in the orchard but as they were common to all treatments and were not applied during the fruiting period, they have not been included in comparison of treatments.

The therapeutants used in these trials for control of bacterial-spot were Bordeaux, lime sulphur and Fermate. Treatments of three replications in 1944-45 and of eight replications in subsequent seasons were randomized, and included plots of six to eight trees of about equal numbers of both Doris and George Wilson varieties. Spray materials were applied from a portable power unit. During the fruiting period the first application of a treatment was given at petal fall which varied with the season from 10th to 20th October. Times of subsequent applications were determined by their number, i.e. they were spaced to provide an even spray cover from petal fall to fruit harvest. Harvesting normally took place between 30th January and 23rd February. All fruits

from each tree were counted and examined, except in 1947-48 when 30 cases of Doris and 38 cases of George Wilson, involving over 13,000 fruits, were taken at random from the orchard during picking. All these fruits were recorded as infected or free from disease.

In the 1944-45 season treatments consisted of unsprayed checks and three, four, five and six applications of 2-6-100 Bordeaux and of 4-6-100 Bordeaux. It was recognized that five or six applications during the fruiting period were more than would be given by growers and for this reason subsequent trials included only four sprays in this period.

In 1945-46 and 1946-47 seasons the trials involved comparisons of unsprayed checks with four applications of Bordeaux and of lime sulphur. From previous trials it was known that foliage damage followed use of 4-6-100 Bordeaux, but as at that time the conditions under which damage occurred were not fully known, the 1945-46 Bordeaux treatments began with 4-6-100 concentration. However, serious foliage damage resulted and spray concentration was reduced as the season progressed. In this season the first Bordeaux application was 4-6-100, the second 3-6-100, the third 2-6-100 and the fourth 2-6-100. Lime sulphur was applied in the first two sprays at 1-50 (1 gal. lime sulphur of 15 per cent polysulphide content to 50 gal. water), but as severe foliage damage occurred the two later sprays were given at 1-80. Five pints of sulphite lye per 100 gal. of spray were included in all applications of both spray materials, and lead arsenate at 1½ lb. in 100 gal. for control of leech and leaf-roller caterpillar was included in the last application. Primarily to determine spray damage effect, two Doris trees were treated with four applications of Fermate at 6 lb. in 100 gal. water, with Agral as a wetting agent at 4 oz. to 100 gal. of spray.

In the 1946-47 season treatments of the previous season were repeated except that concentrations of Bordeaux were 3-6-100 for the first two applications and 1½-3-100 for the two later sprays, and lime sulphur was applied at 1-80 in all four applications. An additional treatment of four applications of Fermate at 6-100 was given to eight George Wilson trees. Agral was included in all applications of Fermate and in all except the first applications of Bordeaux and lime sulphur.

In the last season (1947-48) the orchard was controlled by the owner and treated with Bordeaux only, as follows: Bordeaux 6-8-100 at bud-movement, 2-4-100 for first two bacterial-spot control sprays, and 3-6-100 for third and fourth applications. No unsprayed trees were included in the area. As other spray trials had shown that young growth of Japanese plums was more susceptible than mature foliage to spray damage, the lower concentration of 2-4-100 was applied in the early part of 1947-48 season.

RESULTS

1944–45 season.—In this season, fruit-setting was extremely poor and insufficient fruit was available for analysis of results. A count of all fruit on 29th November when spray programme was not fully completed showed that 4-6-100 Bordeaux gave 2.2, 1.8, 8.2 and 8.8 per cent infection, and that 2-6-100 Bordeaux gave 3.7, 4.4, 12.1 and 22.4 per cent infection for four, three, two and one sprays respectively. Unsprayed fruit showed 33 per cent infection. Prior to harvesting the crop, many trees were partially picked by owner and final results therefore were not available.

TABLE I. EFFECT OF BORDEAUX AND LIME SULPHUR ON OCCURRENCE OF BACTERIAL-SPOT OF PLUM FRUIT

Season.	1945-46.		1946-47.			1947-48.		
Treatment.	Concentration.	Per cent. fruit infected. Doris.	Concentration.	Per cent. fruit infected. Doris.	Per cent. fruit infected. G. Wilson.	Concentration.	Per cent. fruit infected. Doris.	Per cent. fruit infected. G. Wilson.
Bordeaux	Spray 1 4–6–100 2 3–6–100 3 2–6–100 4 2–6–100	6·8	Spray 1 3–6–100 2 3–6–100 3 1½–3–100 4 1½–3–100	4·2	6·8	Spray 1 2–4–100 2 2–4–100 3 3–6–100 4 3–6–100	2·07	2·18
Lime Sulphur	Spray 1 1–50 2 1–50 3 1–80 4 1–80	37·2	all 1–80	23·9	23·3			
Check	No spray	38·9	No spray	19·2	19·9			
Difference for significance at 5 per cent. level		8·7		4·8	6·7			

1945–46 season. Fruit-setting was again very poor and irregular in the George Wilson variety, a total of fifteen hundred fruits of this variety being picked. These gave 5.2, 14.5 and 28.3 per cent infection for Bordeaux, lime sulphur and unsprayed treatments respectively. Results from Doris trees are shown in Table I. Fruit from the two trees sprayed with Fermate averaged 27.7 per cent infection.

1946–47 season. A good crop was obtained from both varieties and results of treatments are given in Table I. Fruits from the Fermate treatment gave an average infection of 29.5 per cent.

1947-48 season. A large crop was produced and only a sample count was made. Doris and George Wilson fruits showed 2.07 and 2.18 per cent infection respectively.

Spray damage of foliage occurred in each season more particularly following the more concentrated applications of both Bordeaux and lime sulphur. The final Bordeaux mixtures in 1947-48 of 2-4-100 for the first two applications and 3-6-100 for later sprays still caused some damage to foliage but not sufficient to be considered serious or to debar the use of Bordeaux. Foliage was not affected by Fermate. In all above trials, fruit was not injured by Bordeaux, lime sulphur or Fermate.

Discussion and Conclusions

The results in Table I show that in each season Bordeaux mixture reduced fruit infection to less than seven per cent, and that lime sulphur did not cause a significant change. The results also show that the amount of infection associated with Bordeaux treatment became less with succeeding years, but it is doubtful if differences are significant. In the final season (1947-48) infection was particularly low. The area had received overall applications of Bordeaux and it is not known whether the low infection was attributable wholly to the efficiency of Bordeaux, or in part to absence of unsprayed checks or possibly to the season being unfavourable to disease development. Reports from other orchards suggest that incidence of bacterial-spot was not less than in previous seasons.

Although Fermate did not cause plant damage it was of no value for control of bacterial-spot.

The results of 1944-45 season suggest that Bordeaux 4-6-100 was better than 2-6-100 and that control was improved with increase in number of applications. These results are in accord with those normally obtained in disease control. Unfortuantely, damage of young plum foliage with Bordeaux 4-6-100 was often severe and necessitated reduction in concentration of the spray material. The final use of 2-4-100 for first two sprays and 3-6-100 for subsequent ones caused very little foliage damage and at the same time provided good control of bacterial-spot.

References

REID, W. D. (1945): Bacterial-spot of Plum and Peach. *N.Z. J. Sci. and Tech., 26,* (*Sec. A*), 359-66.

THE METABOLISM AND TOXICITY OF CYANIDES AND CYANOGENETIC GLUCOSIDES IN SHEEP

III. THE TOXICITY OF CYANIDES AND CYANOGENETIC GLUCOSIDES

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Summary

(1) The M.L.D. of free HCN for sheep is approximately 2.4 mg. HCN/kg. body weight. Starvation for 2-4 days does not significantly alter the M.L.D.

(2) The M.L.D. of free lotaustralin for sheep is 4.5 $\pm$ 0.5 mg. HCN/kg. Starvation for 2-4 days does have a significant effect.

(3) Analysis of body tissues of dead sheep for HCN content is not a very reliable method of diagnosing cyanide poisoning.

(4) By repeated dosing of small amounts of HCN or lotaustralin at rapid intervals it has been shown that sheep can tolerate an hourly intake of up to 3.9 mg.HCN/kg. for several hours on end.

(5) The theory of poisoning by cyanogenetic plants is reviewed in the light of the new evidence presented in Parts I, II and III of this series. The importance of rate of intake and rate of hydrolysis in the rumen is stressed.

Introduction

In certain countries the poisoning of stock by cyanogenetic plants reaches proportions of considerable economic importance. In South Africa a wide range of these plants occurs, causing mortality especially in sheep. This has stimulated a great deal of investigational work by the Onderstepoort workers, who have listed the plants dangerous to stock and have determined many of the factors influencing the toxicity of these plants to stock. American work has resulted largely from the widespread use of Sorghum and Sudan grass in stock feeding, these two species containing cyanogenetic glucosides which may under certain conditions reach toxic proportions. In Australia a considerable number of cyanogenetic plants occurs, including especially Sudan grass. Certain common foodstuffs used all over the world, such as linseed meal, and some clovers, also contain cyanogenetic glucosides and although the incidence of mortality from eating these foods is very low they nevertheless can be important.

Interest in the problem of cyanide poisoning in New Zealand arises mainly from the presence of a cyanogenetic glucoside, lotaustralin, in White Clover (*Trifolium repens* L.). Doak (1) has shown that the most highly producing strains of white clover are those possessing the highest HCN content. Melville *et al.* (2) have discussed the possible toxicity resulting from stock eating highly cyanogenetic pasture and consider that at times stock must consume daily several times the lethal dose of HCN. Corkill (3) has studied the genetic relationships of glucoside and enzyme in regard to the possibility that increase in vigour and productivity of bred strains may be accompanied by an increase in HCN content. It is of interest to know whether, by further breeding and selection for productivity, one is likely to reach toxic levels. On the practical side there is no well authenticated evidence of cyanide poisoning by clover though there have been reports of mortality in sheep going on to clover dominant pastures. Apart from the use of linseed, the only other plant of interest in New Zealand from this point of view is *Eschscholtzia Californica* containing an unidentified glucoside. This plant has limited distribution in the shingly riverbeds of the South Island and is eaten by stock at certain times of the year (4).

While interest in New Zealand is at present of more theoretical than practical importance this may not always be true. Further, any evidence obtained here may be of value in those countries where cyanide poisoning is important.

There is general agreement that the toxicity of HCN administered as KCN per os, and expressed as the minimum lethal dose (M.L.D.) is of the order 2.0 - 2.3 mg. HCN per kg. body weight. Hindmarsh (5) found that 2.2 mg./kg. was lethal when given as an intraperitoneal injection. When given per os, only two sheep were used, one dying after a dose of 2.2 mg./kg. and one surviving a dose of 1.4 mg./kg. Steyn is quoted by Leeman (6) as agreeing with the figure of 2.2 mg./kg. Clawson, Bunyea and Couch (7) state that the M.L.D. of HCN drenched per os is 2.0 mg./kg. for cattle and 2.3 mg./kg. for sheep. Shearer and Sellers (8) found that 2.2 mg./kg. failed to kill two sheep but 3.5 mg./kg. was fatal. While there is reasonable agreement between these results of different authors the numbers of sheep used were small. These dosing trials were therefore repeated in order to determine the magnitude of the M.L.D. and the factors affecting it. These are essential to the interpretation of the toxicity of cyanogenetic plants.

As regards the toxicity of cyanogenetic glucosides and cyanogenetic plants the evidence is somewhat conflicting. There are many instances in the literature, of sheep and cattle succumbing to the ingestion of plant material equivalent to an HCN intake of about 4 mg./kg. but the exact conditions of the animals and of their intakes are unknown. An equally large number of cases have been cited where stock have ingested daily several times the M.L.D. without showing any symptoms of poisoning. Rose (9) records a large number of observations of mortality in cattle following the grazing of Sudan grass. In these observations some cattle put on to Sudan grass quickly succumbed, while others survived even continued grazing of grass very high in HCN content. Such observations are typical of practical aspects of poisoning of sheep and cattle by cyanogenetic plants.

Seddon and King (10) drenched powdered *Acacia glaucescens* and *Eremophilia maculata,* plus powdered almonds to provide enzyme, and found that the M.L.D. expressed as HCN was 2.2 mg./kg., i.e., the same as for free HCN. On the other hand when the isolated glucoside plus almonds was drenched one sheep survived 2.2 mg./kg. and one succumbed to 4.4 mg./kg. The latter observations are difficult to reconcile with those following drenching of the powdered plant. It was not stated whether or not the almonds also contained HCN as glucoside. Franklin and Reid (11) showed that normal sheep could consume the equivalent of 8 - 10 mg.HCN/kg. per day as linseed meal without any mortality. Sheep starved for 5-8 days exhibited a higher susceptibility to poisoning for they showed symptoms of poisoning if they ate linseed at the rate of 2 mg.HCN/kg. in two hours whereas unstarved sheep could eat the same amount without showing any symptoms. Starved sheep force-fed 1.9 - 2.2 mg.HCN/kg. as linseed gruel over a period of 3-4 hours succumbed, whereas unstarved sheep survived. In practice usually only those animals which eat rapidly die, and the evidence of Franklin and Reid supports this in that they had to force feed their sheep to produce any mortality. On their evidence this causes the M.L.D. to approach that of free HCN.

Though some authors believe that chronic cyanide poisoning is possible it is generally recognized that provided free HCN or cyanogenetic plants are ingested at a moderate rate throughout the course of the day animals can tolerate amounts well in excess of the M.L.D. for a single dose. van der Walt (12) failed to produce chronic poisoning in sheep even after administering 3.2 mg.HCN/kg. daily for two years. Worden (13) showed that in the rabbit repeated dosing does not produce a cumulative effect and that the animal is capable of eliminating $\frac{1}{2}$ M.L.D. in $2\frac{1}{2}$ hours. On the other hand there is no evidence that continued sublethal dosing or ingestion causes any resistance or acclimatization to HCN poisoning.

In Part I of this series the rates of release of HCN in the rumen from free glucosides and from cyanogenetic plant material have been measured and the factors influencing these rates determined. Likewise the rates of absorption and the factors affecting the absorption of HCN from the rumen into the bloodstream have been determined. In Part II the probable detoxication mechanism of absorbed HCN is described and it has been shown that *in vitro* liver tissue can detoxicate HCN at a rate equivalent to at least 2 mg.HCN per hour per kg. bodyweight of the sheep. In continuation of this work, and to provide further evidence than that given by the authors already cited concerning the toxicity of cyanogenetic plants the following experiments were conducted:

(i) the determination of the toxicity (M.L.D.) of free HCN and of free cyanogenetic glucosides administered as a single dose to starved and unstarved sheep;

(ii) the determination of the tolerance of sheep to free HCN and free glucosides administered in repeated doses over periods of several hours.

Experimental Material and Methods

Sheep:

In the toxicity trials (Tables I, II, and III) where a single dose was administered to determine the M.L.D. the sheep used were selected from a mob of $6\frac{1}{2}$ year Romney ewes. These ewes were typical in all important respects in that they were normal, healthy, sound mouthed and at the time of the trials (February-May, 1948) they were dry, their lambs having recently been weaned. Before being dosed they were brought in off pasture. This pasture was ryegrass dominant but contained a little clover. The latter was very low in HCN content so that altogether the contribution of HCN from this source could be neglected in relation to the doses given. The weights of the sheep were recorded immediately before dosing, the weight of the starved sheep, for instance, being the starved weight. It was decided to adopt 48 hours' starvation as the most probable length of time that sheep in New Zealand are deprived of food and water in the ordinary trucking, buying and selling of stock. Such a period can scarcely be called starvation in the sense that most people, and Australians in particular, use the term.

For all the tolerance trials, using repeated dosing, the fistulated sheep described in Part I was used. This sheep had not been on pasture immediately prior to dosing but was housed indoors and feed on chaffed lucerne and timothy hays. This sheep was dosed through the fistula.

HCN and Glucoside:

These were administered by means of a stomach tube. Free HCN was given as 0.1 M.KCN, this being followed by about 100 ml. of water. Considering the buffering power and the normal pH values of the rumen it was not considered of any consequence to acidify the KCN though this procedure was adopted for the sheep starved for four days. Cyanogenetic glucoside was given as 5 per cent lotaustralin followed by water. The lotaustralin was 90 per cent pure. No enzyme was given, the results of experiments quoted in Part I showing that it was unnecessary.

Analysis of Organs of Dead Sheep:

Immediately death occurred in any sheep the body was opened and samples taken for analysis. The pH and volume of ruminal contents were measured and the physical consistency noted. Samples of ruminal contents, blood, liver, kidney and muscle were taken for HCN and in some cases thiocyanate analysis. In the case of the organs a sample was taken, frozen rapidly and whilst still frozen solid thin slices were cut, weighed and introduced into the aeration vessel for HCN estimation. Unclotted blood was obtained by rapidly removing the heart and squeezing the blood out into oxalated tubes. HCN and thiocyanate were determined as described in Parts I and II.

Results

Toxicity of HCN administered as a Single Dose

The results obtained are summarized in Table I.

Table I.

Tag No.	Condition of Sheep.	Wt. (kg.)	Dose. (mg. HCN/kg.)	Result.
1	Full stomach	50·0	1·8	Moderate symptoms. Survived
2	,, ,,	50·8	2·4	Severe symptoms. Survived
3	,, ,,	61·3	2·4	Died after 26 mins.
4	,, ,,	52·7	2·7	Died after 34 min.
5	Starved 48 hours	46·2	2·0	Slight symptoms. Survived
6	,, ,,	52·2	2·0	Died after 74 min. (Hydatids)
7	,, ,,	54·9	2·2	Slight symptoms. Survived
8	,, ,,	45·3	2·4	Died after 48 min.
9	Starved 96 hours	47·2	1·93	Moderate symptoms. Survived

The following observations and deductions can be made from these trials:—

(i) The M.L.D. of HCN is approximately 2.4 mg./kg. with a probable error of ± 0.2.

(ii) Starvation for 48-96 hours does not appear to have a significant effect. Sheep No. 6 which succumbed to 2.0 mg./kg. should be excluded as it was found that about half the liver tissue was occupied by hydatid cysts thus reducing its detoxication efficiency.

(iii) The first symptoms were observed as early as 1½ minutes after dosing, confirming the rapidity with which HCN is

absorbed. There was some evidence that the onset of the various stages of poisoning was accelerated in those sheep which had not been starved. Whether this is a true physiological phenomenon is not known. It could be due to the solid mass of ruminal contents in the unstarved sheep preventing dispersion of the HCN, so in effect leaving a pocket of high concentration from which absorption would be unduly rapid.

Toxicity of Lotaustralin Administered as a Single Dose

The results obtained are summarized in Table II.

TABLE II.

Tag No.	Condition of Sheep.	Wt. (kg.)	Dose. (mg. HCN/kg.)	Result.
10	Full stomach	63·0	3·75	Moderate symptoms. Survived
11	,, ,,	56·5	4·8	Died in 54 min.
2	,, ,,	52·6	6·2	Died in 52 min.
12	Starved 24 hours	49·0	3·25	Very slight symptoms. Survived
10	,, ,,	57·2	4·0	Moderate symptoms. Survived
1	Starved 48 hours	49·0	4·0	Slight symptoms after 2 hours. Survived
5	,, ,,	48·0	5·2	Delayed symptoms. Died in 4 hours
10	Starved 96 hours then fed after dosing	48·1	4·0	Delayed symptoms. Died in 4½ hours

The following observations can be made from these experiments:—

(i) The M.L.D. for sheep is 4.5 ± 0.5 mg.HCN/kg. when given as free glucoside.

(ii) There is an indication that starvation for 48 hours reduces the toxicity (increases the M.L.D.) of the glucoside. If one takes into account the relative severity of symptoms as an index of approximation to the M.L.D. it appears that on full stomach the M.L.D. is probably within the range 4.0-4.5 mg./kg. and after 48 hours' starvation it is within 4.5-5.0 mg./kg. The number of sheep dosed is too small to be definite about the point. On the other hand starvation for four days followed by ingestion of food after dosing reduces the M.L.D. to below 4.0 mg./kg. (1 sheep only).

(iii) The symptoms of HCN poisoning are delayed in comparison with the administration of free HCN. The first symptoms appear from 3-7 min. after dosing. On full stomach severe symptoms (falling over, tetany) occur 15-25 min. after dosing in comparison with 5-10 min. with free HCN. Likewise death is delayed.

(iv) The delay in the onset of symptoms is significantly greater with starved sheep. The only symptoms shown by Sheep No. 1 occurred nearly two hours after dosing. In the case of Sheep Nos. 5 and 10 there were no symptoms in the first three hours.

Analysis of Organs of Dead Sheep

In Table III are recorded the ruminal volume and pH, together with the HCN and thiocyanate content of various organs:

Table III. Analysis of Organs of Dead Sheep
(Concentrations in μg./ml.)

Sheep No.	Vol. of Ruminal Contents Litres.	Rumen pH.	Rumen HCN.	Blood HCN.	Liver HCN.	Kidney HCN.	Muscle HCN.	Serum CNS.	Liver CNS.
Dosed with free HCN									
3			0·2	1·2	2·4	0·9	0·7	6·3	
4			0·3	1·0	0·2	0·2	0·7	6·9	
6*	1·8 (v .liquid)	7·33	3·0	1·2	1·2	0·3	0·5	7·1	
8*	3·0 (liquid)	8·03	2·5	1·0	2·0	0·5	0·7	6·8	
Dosed with glucoside									
11	4·5 (solid)	5·74	—	—	—	—	—		
2	3·1 (solid)	6·19	0·7	0·8	2·1	0·4	0·6	11·5	28·9
5*	2·3 (v. liquid)	7·73	3·0	0·9	2·0	0·4	0·6	9·9	29·1
10*	1·0 (moderate)	7·5	6·0	—	3·5	—	—		

* Starved 48 hours or more.

The amount of HCN in the tissues of a sheep poisoned by cyanide can be used as a useful form of diagnosis. This is discussed fully by van der Walt (*loc. cit.*). He concludes that an HCN content of 1.4 μg./g. or more in the liver should generally be regarded as indicating cyanide poisoning allowing that there will always be some biological variation below this level. The levels of HCN in the rumen were more variable but levels above 10 μg./g. could serve as corroborative evidence.

The results presented in Table III agree with those of van der Walt as far as liver is concerned, allowing for some exceptions. The ruminal HCN values are extremely variable and well below the level set by van der Walt. It is evident therefore that this is not a useful guide and there are good reasons for this decision. Death can occur either before or after all the HCN has been absorbed and formation of free HCN from glucoside and its detoxication can still go on in the rumen after death. Actually in the rumen of sheep killed by glucoside no unhydrolysed glucoside was ever detected. The HCN levels in the blood and muscle are the most constant, though in general they are rather low. It was shown in Part II that liver and kidney tissue can bring about rapid detoxication of HCN to HCNS but that blood and muscle produce only a very slow rate of detoxication. It is probable therefore that this accounts for the relative constancy of blood and muscle and the variability of liver and kidney since some time must elapse between death of the animal and the cessation of enzymic detoxication in the tissues.

Samples of liver and kidney kept at room temperature for 24 hours lost nearly all their HCN whilst blood and muscle lost only very small amounts.

In the case of serum thiocyanate the normal level is about 7 μg./ml. (see Part II). The increase due to cyanide poisoning is too small and irregular to be of use in diagnosis. Unfortunately muscle thiocyanate levels were not measured. Liver thiocyanate was measured in only two cases.

Without further work to determine the normal levels of HCN and thiocyanate in the organs of sheep grazing safely on pastures containing cyanogenetic plants it would be unwise to rely on the levels of these substances for diagnostic purposes except as confirmatory evidence. The time that it would take under practical farming conditions to deliver samples from dead sheep to a laboratory for analysis would cause considerable errors. In the case of blood and muscle this may not be true and there may be a case for using these tissues. Unfortunately the safe levels are unknown and even the levels in sheep poisoned by cyanide are so low that experimental error in analysis becomes of considerable importance. Where the normal diet of the sheep does not contain HCN and the tissue levels are therefore normally zero the diagnostic value of HCN levels would be much greater.

One other feature of some importance can be deduced from Table III. Using the known composition of a sheep's body in terms of muscle, fat, bone, blood, etc., and the HCN contents as given in Table III it is probable that the amount of free HCN in the body at death is of the order of 0.5-1.0 mg./kg. This represents therefore the critical level to which HCN content of the body must rise in order to cause mortality.

Toxicity of HCN and Lotaustralin administered in repeated doses

Attempts were made to estimate the toxicity of HCN and lotaustralin when given not as single doses but in repeated sublethal doses. It was necessary to administer doses at frequent intervals and for this purpose the fistulated sheep described in Part I was used. The doses were introduced directly into the rumen via the fistula and conditions within the rumen could be measured at any time by taking ruminal samples. As it was desired not to kill the sheep the tolerance of the animal rather than the toxicity of the HCN and lotaustralin was in fact measured.

Experiment 1:

Repeated doses of HCN. 2nd September, 1947.

The sheep weighed 62 kg. and had been fed during the hour prior to dosing but its rumen was only about half full. Initial pH of the rumen was not recorded but was probably between 6.5-7.0. The pH at the conclusion of dosing was 7.26. HCN was administered as 0.1 M, KCN per fistula. Over a period of 4½ hours from the first dose to the last, the sheep was given 5/6 mg.HCN (= 8.3 mg./kg.). A dose of 75 mg. HCN was given at 30-minute intervals. Immediately before each dose was given a ruminal sample was taken for determination of the HCN content. During the first two hours the HCN content rose as follows: 0.0 μg./ml. (before the trial), 5.4 μg./ml., 8.0 μg./ml., 98 μg./ml., 120 μg./ml. After the fifth dose the sheep fell down and showed moderate signs of poisoning. An interval of one hour was therefore allowed for it to recover after which dosing proceeded, three

more doses, each of 50 mg., being given at 30-min. intervals. The HCN content of the rumen before these three doses was 120 μg./ml., 8 μg./ml., 4.5 μg./ml. respectively and 30 min. after the last dose it was 7.2 μg./ml.

In all 56 mg.HCN was given within 4 hours, or 4½ hours, taking the time to 30 min. after the last dose at which time only 7.2 μg./ml. (or a total of approximately 10 mg.) remained in the rumen. The sheep absorbed from the rumen 516-10 or 506 mg. of HCN over a period of four and a half hours. This is equivalent to an overall tolerance of 1.8 mg.HCN/kg. per hour for four and a half hours.

During the first two hours the sheep could not tolerate the initial rate of dosing, i.e., 2.4 mg/kg. per hour, as was shown by the marked rise in the HCN content of the rumen and the development of symptoms of poisoning. However, in the later stages dosing at 50 mg. per 30 min. or 1.6 mg./kg. per hour was easily tolerated.

Experiment 2:

Repeated doses of HCN. 23rd March, 1948.

The sheep was dosed per fistula with 53.5 mg. HCN at 45 min. intervals for 15 hours. The total amount of HCN given was 1.07 g., equivalent to 15.0 mg./kg. or 1.0 mg./kg./hr. for 15 hours. On occasions immediately after dosing slight panting occurred but otherwise the treatment produced no effect. Ruminal HCN values from samples taken before each dose did not exceed 3 μg.HCN/ml. at any time. The pH remained steady throughout between 6.03-6.20. During the trial the sheep ate food normally.

Experiment 3:

Repeated doses of HCN. 16th April, 1948.

The sheep weighed 74½ kg., had been fed overnight but not fed in the morning just prior to dosing. The initial pH of the rumen was not recorded but the pH at the conclusion of dosing was 7.40. The rumen was only about half full and the contents very fluid and frothy. In this experiment smaller doses at more frequent intervals were given in order to minimize the peak concentrations which occur immediately after any dose is given. It was decided to give an initial large dose to raise immediately the HCN content of the rumen to a level ensuring rapid absorption, this to be followed by smaller doses to maintain this level and hence the rate of absorption.

The initial dose given was 90 mg.HCN (as KCN) followed by a dose of 30 mg. every 5 min. for a period of one hour. Ten minutes from the start the sheep showed a slight increase in breathing rate and from 30 min. onwards it became sleepy and docile—slight symptoms only. At no time were any marked symptoms observed and two hours after dosing the sheep was eating food normally.

The HCN content of the rumen was measured at 15 min. intervals just prior to dosing. It rose progressively as follows: 5 μg./ml., 20 μg./ml., 40 μg./ml., 110 μg./ml. and 15 min. after the last dose it was down to 60 μg./ml. Sampling in between dosing at 5-min. intervals is subject to considerable error due to incomplete mixing in the rumen (see Part I). Too much reliance cannot therefore be placed on these figures other than to show a steady upwards trend to about 100 μg./ml. at the conclusion.

As the HCN content of the rumen was increasing rapidly, dosing at the same rate beyond one hour would certainly soon have produced severe symptoms and death. Therefore 30 mg. every 5 min. or 4.8 mg./kg. per hour, though tolerated for one hour, would eventually prove fatal. Alternatively, if 15 min. after the last dose the HCN content of the rumen was 60 μg./ml. and the rumen volume 1.5 litres there remained in the rumen 90 mg.HCN. Hence the sheep absorbed from the rumen 360 mg.HCN (450 mg. dosed less 90 mg. remaining) over a period of 75 min. which is equivalent to 3.9 mg.HCN/kg. per hour. As the sheep absorbed this amount whilst showing only minor symptoms this value must approximate to the tolerance of the sheep to a continuous intake of HCN.

Experiment 4:

Repeated doses of lotaustralin. September, 1947.

The fistulated sheep was given three doses of lotaustralin at hourly intervals, each dose being equivalent to 100 mg.HCN. The sheep weighed 66 kg., and the pH of the rumen was 6.08. At no time during the experiment were even the slightest symptoms noticed. The HCN levels of the rumen, expressed as μg.HCN/ml., at half hour and one hour intervals after each dose were 0.6 and 0.1, 6.5 and 0.3, 2.5 and 0.2. The rate of dosing is equal to 1.5 mg.HCN/kg. per hour and was much below the tolerance of the sheep.

Experiment 5:

Repeated doses of lotaustralin. 11th May, 1948.

It was hoped to repeat Experiment 2 using glucoside in place of free HCN. Because of the limited quantities of pure lotaustralin available, in both this experiment and Experiment 4 a crude extract was used containing 2 per cent of pure lotaustralin but containing in addition probably 10-20 per cent of soluble sugars and salts. Sugars as explained in Part I cause the lotaustralin to be hydrolysed at a decreased rate, though it was not anticipated that the decrease would be as great as occurred in this experiment.

The fistulated sheep which weighed 76 kg., had been fed before dosing. The initial pH of the rumen was 7.6. The initial dose was 100 mg. HCN (as lotaustralin) followed by doses of 30 mg. every 5 min. for one hour. Thinking the experiment was finished linseed nuts were then fed and in 30 min. the sheep ate an equivalent of 108 mg.HCN. Thus the intake of HCN was 460 mg.HCN in the hour of dosing plus 108 mg. from linseed in the half hour following.

No signs of HCN poisoning were observed until one and a half hours after the first dose at which time the sheep stopped eating the linseed nuts, and showed a general sleepiness for about an hour. It was obvious that the sheep could have tolerated a larger intake of lotaustralin. Ruminal samples were taken for analysis at 15 min. intervals during the first two hours and then at hourly intervals. The HCN content of the rumen expressed in μg.HCN/ml. at times from the initial dose were as follows:—

15 min. - 3.0; 30 min. - 9; 45 min. - 20; 60 min. - 25;
75 min. - 17; 2 hours - 16; 3 hours - 17; 4 hours - 5.0.

Thus over a period of four hours almost the whole of the 568 mg. had been absorbed at an average rate of 1.8 mg/kg. per hour. From the ruminal HCN contents it is evident that over 90 per cent of the HCN is absorbed over the three hours, from half to three and a half hours from the initial dose. This is equivalent to 2.2 mg./kg. per hour.

Discussion

The Theory of Cyanide Poisoning

It has been postulated by several authors that the effect of HCN in the body is to combine with and inhibit the normal functioning of the extra- and intra-cellular respiratory pigments, and/or to damage the central nervous system. Presumably the degree of inhibition or damage must reach a critical level to produce death. This level, using the figures quoted in Table III, is probably of the order 0.5-1.0 mg.HCN/kg. of the body as a whole. The attainment of this critical level is the resultant of two opposing factors—the rate of absorption tending to raise the HCN content of the body and the rate of detoxication lowering it. When the former exceeds the latter to the extent that there is a build up of HCN to the critical level, death ensues.

The absorption of HCN is very rapid and is usually almost complete by the time death occurs. Hence the amount of HCN absorbed in the time up until death can be equated approximately to the M.L.D. Since the critical level is the difference between the amount absorbed and the amount detoxicated the following simplified equation holds—

M.L.D. (HCN) = HCN absorbed = HCN detoxicated + HCN in the body equivalent to the critical level.

This being so, the M.L.D. is affected mainly by the amount of HCN detoxicated in the time up until death and this will obviously be the greater the longer this period is. There are two extreme methods by which the critical level and hence death may be reached. On the one hand a single massive dose of HCN produces a high concentration of HCN in the rumen thereby causing a very high rate of absorption. This exceeds the rate of detoxication to such an extent that HCN accumulates, reaching the critical level in a short time, of the order of 5-20 min. Alternatively, a slower but more prolonged absorption can slightly exceed the detoxication rate so that the critical level is not reached for a period of several hours. In the former case death is caused with the greatest economy of HCN administered, this amount representing the M.L.D. In the case of the latter the amount of HCN administered to cause death is greatly in excess of the M.L.D. for a single dose. This is amply illustrated by the tolerance value of 2-4 mg.HCN/kg. per hour in repeated dosing as compared with an M.L.D. of 2.4 mg.HCN/kg. as a single dose.

The tolerance value is a function of rate of detoxication, and in fact represents the rate of detoxication at the concentration of HCN equivalent to the critical level. In Part II it was shown that the detoxication mechanism consists of the oxidation of HCN by sulphur donors, and H_2S in particular, to yield HCNS. The rate of detoxication varies according to the concentrations of the reactants. Using concentrations equal to those observed in the tissues of animals it was shown that extracted liver tissue is capable *in vitro* of detoxicating HCN at a rate equivalent for the sheep as a whole to 2.1 mg.HCN/kg. per hour. One would expect the rate of detoxication of intact liver *in vivo* to be considerably greater than this. The repeated dosing experiments described in Part III show that *in vivo* the tolerance value or rate of detoxication is as high as 3.9 mg.HCN/kg. per hour under the most favourable conditions, i.e., constant rate of absorption. This raises the question of whether the sulphur donors can set a limit to the tolerance value. Little

data is available on this point. The body contains reserves of cystine and methionine while the food also contributes large quantities (relative to HCN) of these two compounds. H_2S, the most effective reactant, occurs in the rumen in considerable quantities and can be absorbed rapidly. It is probable, therefore, that under normal circumstances the sulphur donors do not limit the rate of detoxication, though this may not be true in sheep which have been starved for many days, where the contribution from the ruminal and intestinal contents is much lower.

The factor of time is now emphasized if figures, albeit approximate figures, for the rate of detoxication and the critical level are introduced into the equation given above—

$$\text{M.L.D.} = 3.9 \text{ mg.HCN/kg./hr.} + 0.75 \text{ mg.HCN/kg.}$$

Thus where the whole of the HCN is absorbed in approximately 20 min. as is the case with free HCN the M.L.D. is of the order 1.3 + 0.75 = 2.05 mg.HCN/kg. If absorption takes place at a slower but more constant rate over a period of one hour (as in glucoside administration) the M.L.D. would be 3.9 + 0.75 = 4.65 mg.HCN/kg. If HCN could be administered evenly and continuously for 12 hours the M.L.D. would be of the order of 46.5 mg.HCN/kg. Provided the detoxication mechanism is not limited by the intake of sulphur donors, the rate of absorption determines the M.L.D.

These observations apply equally well to poisoning by cyanogenetic glucosides. The time required for complete hydrolysis of the glucoside is sufficient to cause a more gradual absorption which the detoxication mechanism is better able to withstand. As shown in Part I a dose of glucoside equivalent to 1.5 mg.HCN/kg. is almost completely hydrolysed within 10-20 min. A lethal dose equivalent to 4.5 mg.HCN/kg. would take longer, perhaps 20-40 min. This slower rate of absorption is the reason for the M.L.D. of free lotaustralin being of the order 4.0-5.0 mg.HCN/kg. Any factor increasing the rate of hydrolysis of the glucoside will lower this value and vice versa. This is confirmed by the observation that the M.L.D. for free lotaustralin dosed to sheep with a full stomach is significantly lower (ca. 4.2 mg.HCN/kg.) than when dosed to sheep on an empty stomach (ca. 4.7 mg.HCN/kg.). In the full stomached sheep bacterial population is high and hydrolysis of the glucoside rapid, whereas in the starved sheep the bacterial population is lower and the pH may be high, consequently hydrolysis is slower.

Where the glucoside ingested is within the intact tissue of the plant the situation is more complex. There is the added complication of the diffusion of glucoside or free HCN out of the ingested plant material and the effect which the plant material itself must have on the pH of the rumen and the bacterial population. It was shown in Part I that in the presence of ruminal fluid the hydrolysis of lotaustralin in dried clover and of linamarin in linseed meal was slightly slower, and the hydrolysis of lotaustralin in fresh clover considerably slower, than that of the free glucoside. Therefore if these foods could be ingested instantaneously in the requisite quantities the M.L.D. would slightly exceed 5 mg.HCN/kg. for dried clover and linseed meal but would considerably exceed this value for fresh clover. As the ingestion of the food takes some time, the M.L.D. must be raised still higher to a value depending on the rapidity with which the food is eaten. The experiments recorded in Part I showed that if dried clover or linseed meal is eaten over a short period, e.g., 15 min., the maximum rate of absorption is reached in some cases within 10-20 min. of the commencement of

eating. By comparison with the hydrolysis of free glucoside this might be equivalent to a M.L.D. of the order of 6 mg.HCN/kg. but it does imply that food containing this amount of HCN is eaten within the 15 min. time limit. In other cases, for instance in fresh clover at intakes equivalent to 1.0 mg.HCN/kg., peak absorption is not reached for 25-30 min. and complete hydrolysis for 60 min. Allowing additional time for the hydrolysis of a larger amount of glucoside as in a lethal dose, the M.L.D. for fresh clover is probably of the order of 7 mg.HCN/kg. Referring back to the equation for the M.L.D. a value such as 7 mg. HCN/kg. implies complete absorption over a period of 100 min.

Where ingestion of food is relatively slow, as in the normal grazing of animals the M.L.D. or tolerance is raised still higher to a value approaching 3.9 mg.HCN/kg. per hour or 31 mg.HCN/kg. over an eight-hour grazing period. Unfortunately the grazing habits of sheep are not accurately known but probably they spend not less than eight hours per day grazing, and allowing for interspersed periods of rumination it is obvious that there is much time during which detoxication can cope with absorption. For this reason grazing animals can tolerate large quantities of HCN. The experiments described in this paper, together with the evidence of Rose, Franklin and Reid. Melville *et al.* (*loc. cit.*) show that animals either dosed or grazing normally and continuously must be capable of tolerating a daily intake of HCN equivalent to 15-50 mg.HCN/kg. Also, since there is continuous slow absorption of HCN the animal is given warning by the appearance of symptoms of poisoning if the rate becomes too high. It then stops eating until this danger period is over whereupon it can recommence eating. These theories and calculations ignore the possibilities of either chronic poisoning or the development of a resistance to cyanide poisoning. There is some evidence that chronic poisoning does occur and that it causes demyceination of the nervous tissue. Hurst (14) found this to be true for monkeys and to a lesser extent for sheep also. On the other hand there is experimental evidence (van der Walt, *loc. cit.*) and much field evidence to show that in practice this does not occur. Likewise there is no evidence that animals can develop a resistance or increased tolerance to HCN by continued ingestion. Sheep taken off cyanogenetic pastures are just as susceptible to dosing with HCN as are those off non-cyanogenetic feeds. The fistulated sheep which had been dosed with HCN and glucoside in large amounts over a period of nearly a year was just as susceptible at the end as at the beginning.

In contrast to the relative safety of slow ingestion, the rapid ingestion of cyanogenetic food is likely to form the most dangerous situation. This has always been recognized and there is much field evidence to substantiate it (van der Walt, Rose, Franklin and Reid, *loc. cit.*). Where animals, often though not necessarily hungry or starved, suddenly gain access to food containing a high content of cyanogenetic glucoside and eat a large quantity in a short time production and absorption of HCN rapidly exceeds the rate of detoxication and death ensues. Provided conditions in the rumen are optimal for bacterial hydrolysis or if ample enzyme is present in the food, the M.L.D. under such circumstances must approximate to 6 mg.HCN/kg. These conditions may not always be as favourable for hydrolysis and the M.L.D. would be correspondingly

higher. One of these conditions is obtained with starved animals. Starvation for 2-4 days or more decreases the bacterial population and increases the pH to between 7.5-8.5. Under these circumstances the rate of hydrolysis is decreased which in turn increases the M.L.D. as has been shown, at least for free glucoside, in this paper. Under practical conditions, however, much plant material is invariably ingested along with the glucoside. While initially hydrolysis may be slow, the presence of food in the rumen brings about a change back to normal pH values and bacterial populations. This causes an ever increasing rate of hydrolysis of the glucoside which may be sudden if the change back to normal is rapid. This probably explains the observation that starved animals (see Table II) can ingest cyanogenetic food lacking in enzyme, appear perfectly composed for several hours, and then suddenly show marked symptoms of cyanide poisoning and die. Other authors have also noted this delayed effect though it is to be expected that such observations would occur less frequently where ample enzyme is present in the plant tissue along with the glucoside. At the same time this phenomenon further increases the dangers of allowing starved animals access to cyanogenetic foods since the delay in onset of symptoms allows them time to ingest toxic amounts before they become disturbed. In antithesis where hydrolysis is extraordinarily rapid, for example, where symptoms occur within 5-10 min. of the commencement of eating, the animal becomes conscious of something abnormal and ceases to eat further. This has been observed in the present work and may account for apparent anomalies cited in the literature of animals showing symptoms but not succumbing when given food normally believed to be toxic.

It is of interest to examine the results of other workers in the light of these observations. Franklin and Reid (*loc. cit.*) observed mortality in a group of starved rams (averaging 54 kg. body weight) when they consumed 0.25 kg. of linseed nuts equivalent to 4.75 mg.HCN/kg. in a single feed but no mortality in unstarved rams consuming 8.0 mg. HCN/kg. Unfortunately the time taken to eat these quantities is not stated and it must be presumed that the starved rams ate their linseed very rapidly and the unstarved ones ate theirs more slowly. The interesting point is that mortality did occur at 4.75 mg.HCN/kg., near the value for free glucoside quoted in this paper. In further trials these authors did not succeed in inducing starved sheep to eat sufficiently rapidly and only produced mortality by force drenching with linseed gruel. In this case a repeated drenching equivalent to 4 mg.HCN/kg. caused mortality in sheep starved for 4-5 days but did not affect unstarved sheep. As already explained this might be due to the accumulation of glucoside in the rumen of the starved sheep until pH and bacterial population changes resulting from the food drenched could bring about rapid hydrolysis. On the other hand it might be due to a real and significant lowering of the M.L.D. due to a more prolonged starvation than was carried out in our work.

Rose (*loc. cit.*) observed that cattle could be maintained continuously and with impunity on Sudan grass in seed containing as much as 0.133 per cent HCN. The dry matter content of the feed was not stated but assuming it to be 50 per cent, and appetite in dry matter to be 2 per cent of body weight on such feed, the daily intake of HCN would be approximately 50 mg./kg. On the other hand there was heavy mortality in cattle put on to Sudan grass for an hour or so each day when the HCN content was as low as 0.07 per cent on a dry matter basis. If a 350 kg. cow

ate 3.0 kg. of dry matter in 15 min. this is equivalent to 6.0 mg.HCN/kg. which is in line with theory provided the cow does eat this amount of food in 15 min.

The amount of food eaten in these cases of mortality is often a critical factor, but unfortunately it is never measured. Our records of stall-fed animals have shown on occasions an intake in 15 min. for a 50 kg. sheep of as much as 0.6 kg. of fresh pasture and 0.45 kg. of dry feed. The capacity of the rumen of a 50 kg. sheep is of the order 3-4 litres. The daily intake of sheep in the field is usually considerably greater than that of sheep fed in pens. It is likely therefore that the rate of intake of sheep grazing undisturbed in the field would exceed the values quoted. It is quite feasible for the animals quoted by Rose and Franklin and Reid to eat the equivalent of 5-6 mg.HCN/kg. within 15 min.

That such amounts should be toxic also assumes rapid liberation of the HCN. This is undoubtedly true for linseed and for Sudan grass considering the rapidity with which symptoms occur. It has already been shown in Part I that the presence of enzyme in the food is not essential for hydrolysis of the glucoside, but with certain foods it is undoubtedly more important than the ruminal bacteria. With dried clover and linseed the presence of enzyme is of little advantage since the glucoside is either free or readily diffuses out of the tissue, but with fresh green tissue such as wet clover a considerable amount of enzymic hydrolysis takes place before diffusion of glucoside out or ingress of bacteria into the tissue occurs. It is therefore very probable that the release of HCN from fresh green tissue will be more rapid if it contains enzyme as well as glucoside. This must be taken into account when estimating the possible toxicity of clover as eaten by sheep and cattle.

From the data presented in Part I it is likely that the ingestion of 7 mg.HCN/kg., as fresh clover, in 15 min. would be fatal. If a 50 kg. sheep can eat a maximum of 0.9 kg. in this time the above fatal dose will occur if the HCN content of the clover is 0.039 per cent and if enzyme is also present. This is in rough agreement with the observations of Rose, Bunyea *et al.* (*loc. cit.*). Since in a pasture, clover is not likely to form more than 60 per cent of the total food ingested, this allows the HCN content to be increased to 0.065 per cent. Further, since the leaf blades contain about three times as much glucoside as do the petioles, and these leaf blades represent about 60 per cent of the ingested clover, the toxic level based on the HCN content of clover leaf blades would be 0.09 per cent. The daily intake of HCN on such a pasture would be 50-60 mg./kg., if the 50 kg. sheep ate seven and a half kg. of fresh pasture (equals one and a half kg. dry matter). On the score of daily tolerance, clover containing as much as 0.09 per cent HCN must also be considered very dangerous. After allowing for a slight safety margin it would be unwise to breed clover strains containing more than about 0.07 per cent HCN based on the leaf blade content. The maximum value recorded so far is 0.05 per cent.

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REFERENCES

(1) DOAK, B. W. (1933): *N.Z. J. Sci. and Tech., 14,* 359.
(2) MELVILLE, J., COOP, I. E., DOAK, B. W. and REIFER, I. (1940): *N.Z. J. Sci. and Tech., 22,* 144 B.
(3) CORKILL, L. (1942): *N.Z. J. Sci. and Tech., 23,* 178 B.
(4) ANON. (1947): *N.Z. J. Agr., 75,* 181.
(5) HINDMARSH, W. L. (1930): *J. Counc. Sci. Ind. Res. Aust., 3,* 12.
(6) LEEMAN, A C. (1935): *Onderstepoort J., 5,* 97.
(7) BUNYEA, H., COUCH, J. F. and CLAWSON, A. B. (1934, 1935): *J. Wash. Acad. Sci., 24,* 523; *25,* 272
(8) SHEARER, G. D. and SELLARS, K. C. (1944): *Vet. J., 100,* 92.
(9) ROSE, A. L. (1941): *Aust Vet. J., 17,* 211.
(10) SEDDON, H. R. and KING, R. O. C. (1930): *J. Counc. Sci. Ind. Res. Aust., 3,* 14.
(11) FRANKLIN, M. C. and REID, R. L. (1944): *Aust. Vet. J., 20,* 332.
(12) VAN DER WALT, S. J. (1944): *Onderstepoort J., 19,* 79.
(13) WORDEN, A. N. (1940): *Vet. Records, 52,* 857.
(14) HURST, E. W. (1940, 1942): *Aust. J. Exp. Biol. and Med. Sci., 18,* 201; *20,* 297.

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Some months ago the British Standards Institution announced that a technical committee had been appointed to devise simple, common names for well established pest control products (including insecticides, insect repellants, acaricides, nematocides, fungicides, herbicides, and rodenticides) in order to help farmers, fruitgrowers, market gardeners and others in the simple identification of these products.

This technical committee, working in collaboration with standards organizations in New Zealand and the other Commonwealth countries and also U.S.A., has now prepared the first draft list containing some 25 common names for the better-known products now in general use in agriculture and veterinary circles throughout the country.

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Further details on this project will be supplied on application to the Director, N.Z. Standards Institute, P.O. Box 3049, Wellington.

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